

## Research Article

# Effect of Storage Time and Packing Method on the Freshness of Dried *Lycium* Fruit Using Electronic Nose and Chemometrics

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The effect of storage time and packing method on dried *Lycium* fruits was studied through an electronic olfactory system with the metal oxide sensor array that provides an overall perception of the volatile compounds presented in the sample headspace. Principle component analysis (PCA), canonical discriminant analysis (CDA), and cluster analysis (CA) were used for freshness and packing methods discrimination of dried *Lycium* fruits. The stale samples of 2015 and 2016 could be separated with those of 2017 by PCA, CDA, and CA analysis. Better discrimination results were obtained by CDA, with samples of 2015 and 2016 separated with each other. For samples of 2017, the unpackaged samples of 2017-4 were distinguished with the vacuumed samples, while samples of grade C were separated with B and D. For quantitative analysis, predictive models for prediction of the storage years of dried *Lycium* fruits were built with methods of partial least square (PLS) analysis, multiple linear regression (MLR), and back propagation neural network (BPNN). The model built by BPNN showed the best predict ability with  $R^2 = 0.9994$ , while PLS and MLR were also effective in the prediction of storage years of dried *Lycium* fruits, with high determination coefficients of 0.9316 and 0.9330. These findings showed that E-nose can be used in the discrimination of the storage time and package method of dried *Lycium* fruits.

## 1. Introduction

*Lycium barbarum* L., a Solanaceae defoliated shrubbery that grows in China, Tibet, and other parts of Asia, reportedly have many health-enhancing effects, such as immune modulation, antioxidant effects, and eye health benefits [1, 2]. The dried fruits have been used as traditional medicine in China and in many countries for more than 2500 years [3].

However, the content of the bioactive substances in dried *Lycium* fruits declines with storage, such as the content of *Lycium barbarum* polysaccharides [4], chlorogenic acid, [5, 6], and total flavonoids [7], indicating that stale *Lycium* fruits weaken in terms of pharmacodynamic effects. It is very difficult for customs to recognize the freshness of dried

*Lycium* fruits. Guaranteeing the quality of commercialized products of *L. barbarum* is important.

For the quality detection of dried *Lycium* fruits, sensory evaluation [8], physical and chemical component analysis and fingerprint technology, and spectroscopy technology are used. For sensory evaluation, it is the traditional method used for quality differentiation. However, it relies on the specialists and unable to give the content of internal components, which limits the application. For methods based on physical and chemical component analysis and fingerprint technology [9], they were effective in regional distribution [10–12], variety identification, and differentiation [13, 14]. The complex sample pretreatment, long detection cycle, and effects by external conditions could not be ignored. For spectroscopy technology, it is widely used for tracing the

origin of *L. barbarum* [15, 16] varieties, and species differentiation of eight *Lycium* species with two-dimensional infrared spectroscopic fingerprinting have been studied [17]. However, samples were usually crushed to powder [16] to guarantee the sample homogeneity and spectral data stability. Thus, it was not a nondestructive test. The methods used to detect the quality of dried *Lycium* fruits could not meet the requirement of market supervision department for fast and nondestructive detection methods. It is important to develop a fast, nondestructive method to distinguish and predict the freshness of dried *Lycium* fruits.

The difference in the composition and content of inner components of dried *Lycium* fruits results in varied odors of volatiles on the surface, thereby providing a basis for the nondestructive internal quality assessment of dried *Lycium* fruits by using the information of volatile compounds. However, the composition of volatiles is complex and difficult to detect and analyze. Works conducted mainly focused on the composition and content of volatile compounds for Goji berry of different origins [18] and processing methods [19]. Few studies were conducted on the judgment of storage years and other factors on the freshness by using flavor compounds.

Gas sensor arrays are sensitive to the volatiles of samples and give a comprehensive odor fingerprint, which could be used for qualitative and quantitative analysis of the non-destructive freshness judgment of dried *Lycium* fruits. Detection of the volatile odors released from the whole-dried *Lycium* fruits allows the development of a fast, objective, and nondestructive method for rapid evaluation of the freshness of dried *Lycium* fruits in the market. Electronic nose (E-nose) is a device developed for sensing of odor information by using a gas sensor array that is sensitive to volatile compounds released from the whole sample. With the advantages of simple sample preparation, high sensitivity, reliability, and repeatability, E-nose has been widely used in food and agricultural products for quality control, environmental monitoring, medical diagnosis, explosive detection, and other fields.

Preliminary studies on E-nose have been conducted on agricultural products used for medicine and food. The E-nose was used for variety differentiation and identification. It was used for determination of the difference between American and Asian ginseng [20], as well as variety differentiation of three cultivars of *Perilla frutescens* [21], 10 different varieties of Chinese jujubes [22], and 13 species of Chinese medicine ([23]. For origin traceability, *Radix Angelica sinensis* (Danggui) from 4 different producing areas were distinguished by the E-nose [24]. In addition, raw samples of Licorice roots were classified according to their origin with the help of the E-nose [25]. For the identification of processing methods for products used for medicine and food, differences between fresh and steamed *Arctium lappa* L. [26] and stir-fried processed *Crataegus* in different degrees [27] were effectively determined by the E-nose. For quantification prediction, the correlation between the *Eurycoma longifolia* extract content and E-nose response was studied [28], and an effective predictive model for rapidly identifying total glucosinolate content in Maca was built

[29]. Moreover, coupled with back propagation-artificial neural networks, the E-nose showed high ability to predict ginseng age [30].

Recently, the E-nose was used to identify production areas of Zhongning goji berries [31]. However, the smell of dried *Lycium* fruits was seldom employed for freshness detection, and few studies were conducted on the correlation between E-nose sensor responses and the freshness of dried *Lycium* fruits.

Dried *Lycium* fruits that differed in freshness were detected using the E-nose to do the following: (1) to evaluate the changes in odor of *Lycium* fruits due to differences in freshness; (2) to analyze the correlation between odor and freshness of *Lycium* fruits; and (3) to provide references and establish a new method for market surveillance of dried *Lycium* fruits.

## 2. Materials and Methods

**2.1. Dried *Lycium* Fruit Samples.** The fruits of cultivated *L. barbarum* were dried naturally immediately after picking. The dried *Lycium* fruits were collected from June to September in 2015, 2016, and 2017 in Zhongning, Ningxia Hui Autonomous Region, China. As listed in Table 1, sample 1 was produced in the year 2015, sample 2 was produced in the year 2016, and samples 3–6 were produced in the year 2017 with different grades. The samples were stored for 3, 2, and 1 years, respectively. Each kind of dried *Lycium* fruits were sealed in two zipper bags, and then stored at 4°C in the refrigerator to prevent moisture absorption and softening of the dried fruits.

All the dried *Lycium* fruit samples were brought to room temperature before E-nose detection. In addition, 31 duplications were prepared for each kind of samples. 1~2 responses were eliminated as the E-nose needs time to adapt to a new sample.

**2.2. Electronic Nose (E-Nose).** To obtain the odor of volatiles emitted from dried *Lycium* fruits, an E-nose of PEN2 (Airsense Corporation, Germany) was used. The E-nose system consists of three parts, namely, a sensor array, a detector unit, and a pattern recognition system. The nomenclature and characteristics of the 10 metal oxide sensors are listed in Table 2, showing that each sensor has a certain degree of affinity toward specific chemical or volatile compounds. The software Win Muster v.1.6 was used for data recording.

In our former study, the experimental conditions for the detection of dried *Lycium* fruits by the E-nose were optimized [32] and given as follows. Before detected by the E-nose, the dried *Lycium* fruit samples were brought to room temperature. The headspace used for the detection of E-nose was generated by the following processes. Without any preparation, 20.0 g of the dried *Lycium* fruits was placed in a beaker (500 mL). The beaker was sealed by a plastic and kept for 30 min at room temperature of 25°C ± 3°C for the headspace to stabilize. During sampling, the stabilized headspace was transferred into the sensor chamber at a flow

TABLE 1: The profiles of dried *Lycium* fruits samples.

No.	Samples	Producing year	Granularity (seed/50 g)	Package method	Storage time (years)	Dry weight of 100 seeds (g)	Grade (seeds/50 g)
1	2015	2015	396 ± 13	Vacuum packaged	3	12.4 ± 0.75	C (≤580)
2	2016	2016	386 ± 22	Vacuum packaged	2	13.19 ± 0.51	C (≤580)
3	2017-1	2017	392 ± 3	Vacuum packaged	1	12.55 ± 0.45	C (≤580)
4	2017-2	2017	328 ± 2	Vacuum packaged	1	15.09 ± 0.78	B (≤370)
5	2017-3	2017	663 ± 4	Vacuum packaged	1	7.67 ± 0.30	D (≤900)
6	2017-4	2017	425 ± 5	Bulk packaged	1	11.89 ± 0.30	C (≤580)

TABLE 2: Sensors used and their main applications in PEN 2 electronic nose.

Number in array	Sensor name	General description	Reference
S1	W1C-aromatic	Aromatic compounds	Toluene, 10 ppm
S2	W5S-broad range	Very sensitive, broad-range sensitivity, react on nitrogen oxides, very sensitive with negative signal	NO <sub>2</sub> , 1 ppm
S3	W3C-aromatic	Ammonia, used as sensor for aromatic compounds	Benzene, 1 ppm
S4	W6S-hydrogen	Mainly hydrogen, selectively (breath gases)	H <sub>2</sub> , 100 ppb
S5	W5C-arom-aliph	Alkanes, aromatic compounds, less polar compounds	Propane, 1 ppm
S6	W1S-broad-methane	Sensitive to methane (environment) ca, 10 ppm, broad range, similar to no.8	CH <sub>4</sub> , 100 ppm
S7	W1W-sulfur-organic	Reacts on sulfur compounds, H <sub>2</sub> S 0.1 ppm; otherwise, sensitive to many terpenes and sulfur organic compounds, which are important for smell, limonene, and pyrazine	H <sub>2</sub> S, 1 ppm
S8	W2S-broad-alcohol	Detects alcohols, partially aromatic compounds, broad range	CO, 100 ppm
S9	W2W-sulph-chlor	Aromatic compounds, sulfur organic compounds	H <sub>2</sub> S, 1 ppm
S10	W3S methane-aliph	Reacts on high concentration >100 ppm, sometime very selective (methane)	CH <sub>4</sub> , 100 ppm

rate of 300 mL·min<sup>-1</sup>. The detection procedure was as follows. For each sample, the measurement time was set to 70 s at an interval of 1 s, and the sensors were rinsed for 80 s with clean air before the detection of the next sample. All the samples were detected at room temperature with 31 duplications.

**2.3. Granularity and Dry Weight of 100 Seeds.** The granularity and dry weight of 100 seeds, indicating the seed size, maturity, and the quality, were detected according to the national standards of [33], and the results are listed in Table 1.

**2.4. Statistical Analysis.** The ability of the E-nose in the evaluation of dried *Lycium* fruits freshness was judged by the chemometrics. Principle component analysis (PCA), canonical discriminate analysis (CDA), and cluster analysis (CA) were employed to visualize the difference of dried *Lycium* fruits with different freshness. To study the correlation between the storage years of the dried *Lycium* fruits and E-nose sensor responses, multiple linear regression (MLR) with the step regression method, partial least square (PLS) regression with cross-validation, and back propagation neural network (BPNN) were performed. And the predictive capacity of the E-nose for the storage years of

dried *Lycium* fruits was compared. Both the qualitative and quantitative methods were compared to find the better one.

For data analysis, the SAS version 8 (SAS Institute Inc., Gary, USA) was used. The figures were plotted with Origin Pro 8.

### 3. Results and Discussion

**3.1. Sensor Array Responses to Dried *Lycium* Fruits.** The typical responses of E-nose sensors to dried *Lycium* fruits produced in the years 2015, 2016, and 2017 are shown in Figure 1. For one sample, each curve represents a sensor transient. For the E-nose, the output signal of each sensor is given as G/G<sub>0</sub>, where G<sub>0</sub> represents the electronic conductivity (resistance) of sensor to the zero gas, which is ambient air after filtered by active carbon, and G represents the electronic conductivity (resistance) of the sensor to the sample gas.

After an initial period of low and stable conductivity, the conductivities for sensor S2 increased sharply within the first 30 s and then stabilized after 50 s of collection time. The conductivities of sensor S6, S7, S8, and S9 increased slowly during detection. For sensors S1, S3, and S5, the conductivities dropped slightly and stabilized after 40 s collection time. The responses of S4 and S10 to dried *Lycium* fruits were

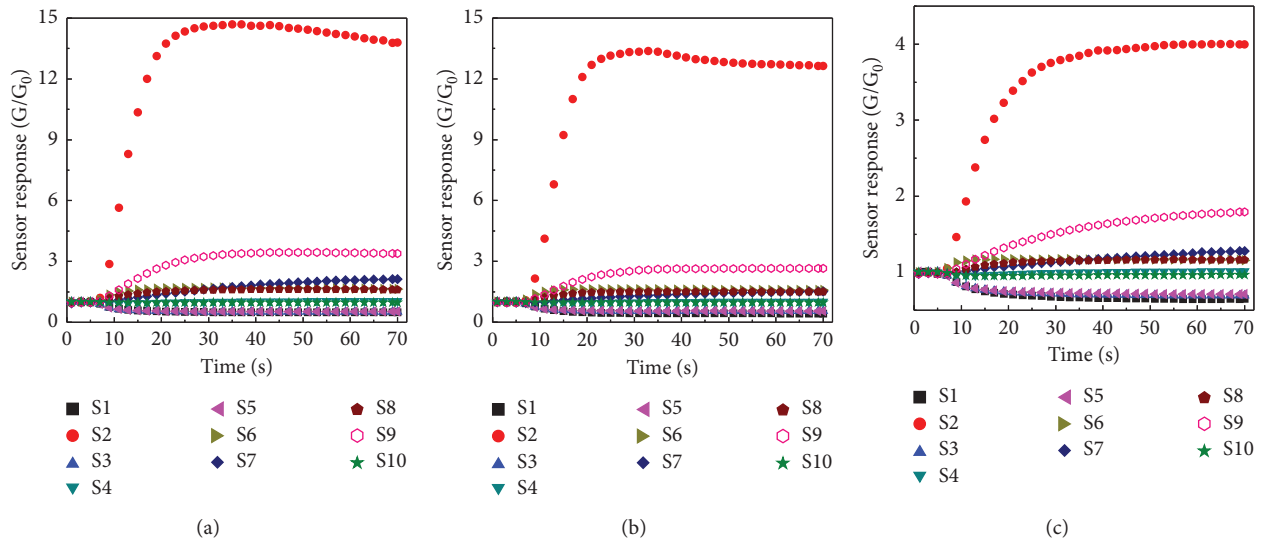


FIGURE 1: Ten sensors' response curve to the dried *Lycium* fruits samples with different producing years.

weak. The sensor signal stabilized generally and was considered for further analysis.

As shown in Figure 1, the response tendency of the sensors was quite similar for dried *Lycium* fruits produced in different years, whereas the response varied greatly in intensity for S2 and S9.

The stabilized sensor responses at the 70<sup>th</sup> second were extracted and analyzed by ANOVA, and the results are shown in Table 3. As shown in Table 3, except for S4, the responses of sensors S1–S3 and S5–S10 were significantly affected by the producing year of dried *Lycium* fruits, thereby laying the foundation for the differentiation of dried *Lycium* fruits produced in different years.

**3.2. Discrimination Storage Years and Packing Method of Dried *Lycium* Fruits with PCA and CDA.** For the volatile components of dried *Lycium* fruit, it is reported that the nitrogenous substances, such as 6-methyl-6-nitroheptan-2-one, made the responses of S2 changed greatly. For the high content of aldehydes, such as hexanal and 2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde, alcohols of 1-octen-3-ol, and ketones of 2,3-butanedione, makes the responses of S8 and S6 differed with each other. For the major aromatic compounds of alkanes, furan, pyrazines, and aromatic ether, the sensors S1, S3, S5, S9, and S10 respond to these flavors. For terpenes, 10 compounds were reported, which made the response of S7 changes. Furthermore, hexanal and 1-octen-3-ol, the oxidation products of fatty acids, and 2, 6, 6-trimethyl-1, 3-cyclohexadiene-1-carboxaldehyde, the oxidation products of carotene components, were the prime candidates of undesirable odor of dried *Lycium* fruits and its products. During the storage of dried *Lycium* fruits, the smell changed due to the oxidation, hydrolysis of carotene components,  $\omega$ 5 group of the C18 fatty acids, and other components [9]. All these changes of volatile compounds were detected by the E-nose.

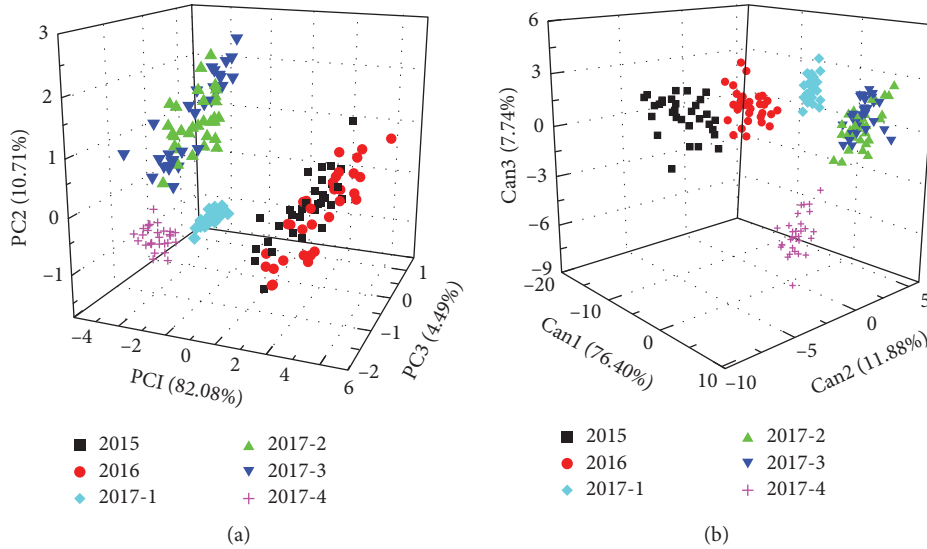
The extracted sensor responses of the E-nose to dried *Lycium* fruits at the 70<sup>th</sup> second were analyzed by PCA and CDA, and the results are shown in Figure 2. In Figure 2(a), the first three PCs (PC1, PC2, and PC3) explained 97.28% of the total variance, providing most of the odor information of dried *Lycium* fruits. Good separation in the PC1 direction could be found for fresh *Lycium* fruits and stale samples. The stale samples of 2015 and 2016 could be separated with that of 2017 with PCA. Samples produced in 2015 and 2016 overlapped with one another and scattered in the right of the plot, with  $PC1 > 0$ . Samples produced in the year 2017 scattered in the left part, with  $PC1 < 0$ . Samples of grades B and D overlapped with each other, they could be separated with that of grade C. For samples of grade C, different package affected the freshness of dried *Lycium* fruits, and samples of 2017-1 (vacuum packaged) and 2017-4 (bulk packaged) were separated.

With PCA, the stale fruits and different grades of *Lycium* fruits can be discriminated; the same results were obtained by the taste information obtained by the taste sensing system with artificial lipid-based membrane sensors in our former studies [34]. The ability of the E-nose to discriminate stale-dried *Lycium* fruits from fresh ones provided a new non-destructive method for the detection of adulteration of dried *Lycium* fruits with stale ones.

The CDA results were shown in Figure 2(b). Can1, Can2, and Can3 explained 96.02% of the total variance with the value of 100%. Thus, the first three CANs can give most information of the E-nose data set. The samples were grouped into three clusters according to the producing year. The stale samples of 2015 and 2016 could be separated with that of 2017 with CDA. And the 2015 and 2016 samples were discriminated from each other, and results were better than those of PCA. For samples produced in the year 2017, the CDA results were similar with that of PCA. With different freshness of dried *Lycium* fruits, the smell changed with the producing year. This observation could be used in the

TABLE 3: Results of ANOVA.

Factors		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Producing year	F	425.74	544.96	379.27	0.8	338.29	348.68	40.40	420.24	125.96	216.66
	Sig.	<0.0001	<0.0001	<0.0001	0.4984	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

FIGURE 2: (a) PCA and (b) CDA scatter plot of dried *Lycium* fruits produced in different years based on E-nose responses.

discrimination of adulteration of dried *Lycium* fruits with stale ones.

**3.3. Classification of Storage Years and Packing Method of Dried *Lycium* Fruit with CA.** For dried *Lycium* fruits, CA was conducted based on odor information. The dendrogram obtained by CA is shown in Figure 3.

In Figure 3, the results obtained by CA showed that, at the distance  $D = 1.3711$ , the samples of dried *Lycium* fruits were clustered into two groups, namely, cluster 1 (the stale samples of 2015 and 2016) and cluster 2 (the four samples produced in 2017). For cluster 1, samples of 2015 and 2016 could not be discriminated. For cluster 2, the samples of 2017-1 and 2017-4 were discriminated with others at the distance of 0.7301. However, 2017-2 and 2017-3 could not be distinguished from each other, similar to the PCA results.

With sensor responses, the E-nose system could recognize the fresh and stale fruits of dried *Lycium* fruits by PCA, CDA, and CA methods.

**3.4. Rapid Characterization of Storage Years of Dried *Lycium* Fruits.** To establish the relationship between the responses of E-nose and storage years of dried *Lycium* fruits, analytical methods of MLR, PLS, and BPNN were used, and the results were compared to find the best predictive model.

Data set containing dried *Lycium* fruit samples (156 for calibration and 22 for validation) was used to build the predictive model for the storage years of dried *Lycium* fruits. The correlation coefficient ( $R^2$ ) and root mean square error (RMSE) between predicted and experimental values (RMSE)

were used to evaluate the model performance. Large  $R^2$  and low RMSE lead to good calibration model. The results are shown in Figure 4 and Table 4.

With the stepwise method, the MLR algorithm established a model that describes the relationship between the E-nose sensor signals and the storage years of dried *Lycium* fruits. The predictive models for storage years of dried *Lycium* fruits were given as follows.

$$\text{Storage years of dried } Lycium \text{ fruits} = 9.873 \times S1 - 31.187 \times S3 + 19.800 \times S5 - 3.476 \times S7 + 2.576 \times S9 + 12.132 \times S10 - 9.506.$$

Large  $R^2$  and low RMSE indicate adequate fits. As shown in Table 4, the predictive ability of the E-nose was listed. A linear correlation was observed between the responses of E-nose sensors and the storage years of dried *Lycium* fruits. When the model was used to predict the storage years of dried *Lycium* fruits in the test data set,  $R^2$  was 0.9239, thereby showing good ability to predict the storage years of dried *Lycium* fruits.

For models built by PLS, the coefficient  $R^2 = 0.9316$  was found between the E-nose sensor responses and storage years of dried *Lycium* fruit samples. When the model was used to predict samples in the test set,  $R^2$  of 0.9480 was obtained. For the prediction of storage years of dried *Lycium* fruits, the PLS methods are slightly better than that of MLR.

For BPNN, the experimental design was completely randomized with each sample as an experimental unit. The architecture of the chosen artificial neural network was  $N \times (2N + 1) \times M$  three-layer back propagation. The input layer, i.e., one hidden layer, was designed as 10 neurons according to the sensor array of 10 sensors. The output layer

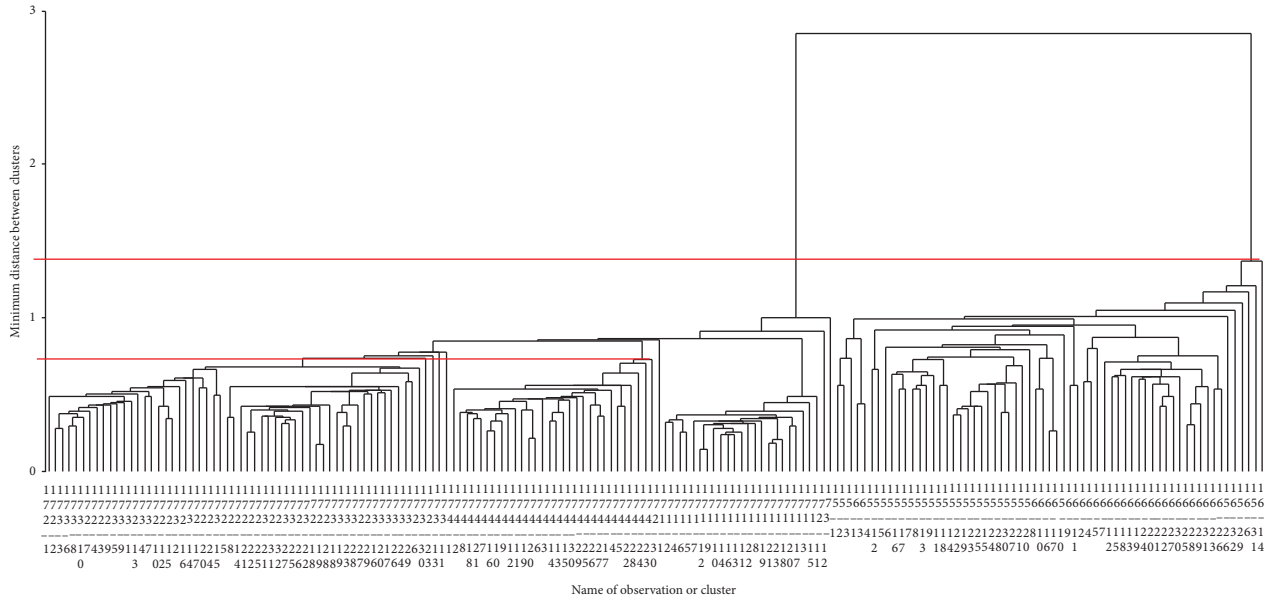


FIGURE 3: Tree diagram from CA by minimum distance with smell information.

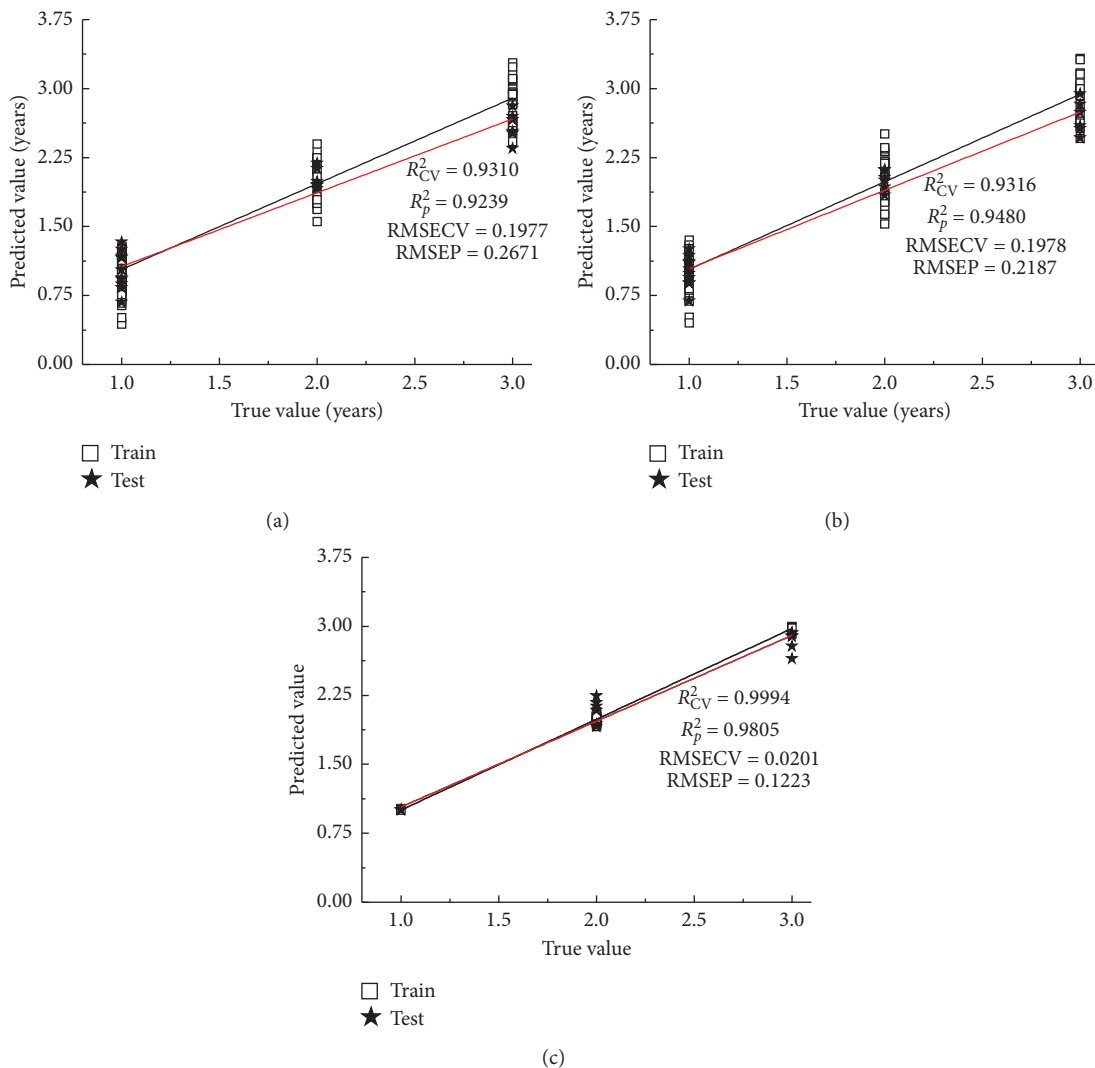


FIGURE 4: Prediction storage years of dried *Lycium* fruits by MLR, PLS, and BPNN.

TABLE 4: Comparison of three quantitative prediction methods for storage years of dried *Lycium* fruits.

Prediction methods	Training set		Test set	
	$R_{cv}^2$	RMSEC (%)	$R_p^2$	RMSEP (%)
MLR	0.9310	0.1977	0.9239	0.2671
PLS	0.9316	0.1978	0.9480	0.2187
BPNN	0.9994	0.0201	0.9805	0.1223

had three neurons for different storage years of *Lycium* fruits.

The results obtained by the training model of BPNN showed that all the training and testing samples were correctly classified according to the storage years of *Lycium* fruits. Moreover, the correlations between the observed and predicted storage years of dried *Lycium* fruits were higher than 0.98 (Table 4) for the training and testing sets. The low errors of prediction and the high correlation of the BPNN model suggested that the E-nose can be successfully applied to the determination of the storage years of dried *Lycium* fruits. The BPNN-E-nose methods were suitable for the prediction of storage age for wheat [35–37].

In conclusion, the best results were obtained by the BPNN model, with high correlation (higher than 0.98) for the training and testing subsets.

For the E-nose, it has shown its ability in the discrimination of dried *Lycium* fruits for the freshness and the packing methods and also in the prediction of storage years. Former studies were conducted on the *Lycium ruthenicum* Murr., for producing area, species, and drying conditions [38]. In the future, more applications of the E-nose on quality detection should be studied to give more objective results.

#### 4. Conclusions

Multivariate techniques (PCA, CDA, and CA) were used to prove the E-nose's usefulness for discriminating dried *Lycium* fruits according to freshness. Among the three methods, CDA was suitable for the freshness differentiation of dried *Lycium* fruits according to the producing year. Strong correlation was observed between the E-nose response and freshness of dried *Lycium* fruits, and BPNN was the most suitable for the prediction of storage years. The results obtained by this study are promising in terms of further development of rapid and nondestructive detection methods based on E-nose systems for discrimination between dried *Lycium* fruits and stale ones.

#### Data Availability

The research data about this manuscript used to support the findings of this study are included within the article.

#### Disclosure

This study has used the E-nose for nondestructive detection of dried *Lycium* fruit freshness, which provides a method to evaluate the quality of *Lycium* fruit.

#### Conflicts of Interest

The authors declare that they have no conflicts of interest.

#### Authors' Contributions

M. L. and Y. L. carried out experimentation; X. T. and Z. W. were responsible for data analysis; X. T. and J. W. acquired funding. X. T. and Z. M. investigated the study; S. C., P. Z., X. B., L. S., and L. L. wrote, reviewed, and edited the manuscript.

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#### Supplementary Materials

Graphical Abstract (*Supplementary Materials*)

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