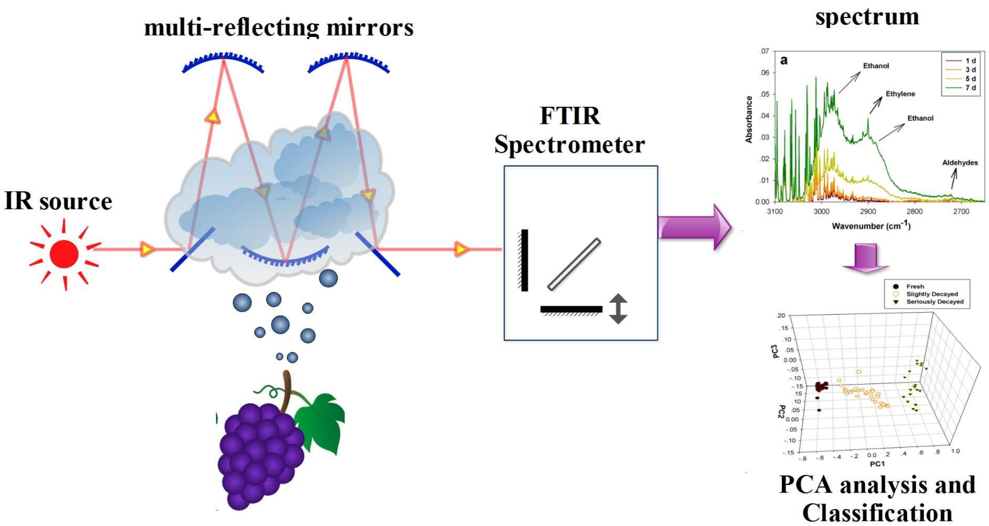

Infrared spectroscopy and sensor array had been use to differentiate the grapes in different spoilage stages *via* their volatiles.

Analysis and Discrimination of Grape Spoilage via Volatiles: A Comparison between Long Optical Path Fourier-transform-infrared Spectroscopy and Sensor Arrays

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Abstract:

Fruits release specific volatiles as vapors during spoilage that can provide information about the spoilage stages of fruits. We used long optical path Fourier Transform Infrared Spectroscopy (FTIR) and sensor arrays comprising carbon dioxide and ethanol sensors to study the grape spoilage process synchronously. The results revealed that specific volatiles, such as carbon dioxide, ethanol and esters, are released from grapes during spoilage. The presence and concentrations of these compounds gradually changes with storage time. Through chemometrics analysis, the infrared spectra of volatiles from different spoilage stages of grapes were successfully classified. As a simple form of instrumentation, the sensor arrays also have the ability to discriminate whether the grapes have decayed. We established a Soft Independent Modeling of Class Analogy (SIMCA) model to classify the grape samples into different spoilage stages, and the model according to different quantities of grapes is also discussed. This study demonstrates that it is possible to characterize grape spoilage by analyzing the released volatiles.

Keywords: volatiles; FTIR; grape; PCA; SIMCA

1. Introduction

Grapes are a popular fruit worldwide, and they are also the main raw material for juice and wine production[1]. However, grapes readily decay at room temperature. The analysis, monitoring and prediction of grape spoilage are of great importance. Furthermore, the monitoring of the grape fermentation process is particularly important in wine production[1].

The volatiles vaporized from vegetables and fruits vary in composition and concentration depending on the quality[2], freshness [3], storage environment[3-6], harvest maturity[7, 8], surface processing methods[9] and on the specific microorganism composition and respiration[10]. These volatiles can thus be used in food quality analysis[11]. Researchers have collected and analyzed the volatiles from grapes and found that they include alcohols, esters, aldehydes and carbon dioxides[12-15]. The concentrations of the above gases were found to vary with the grape variety, storage conditions, ripeness and surface processing methods as well as with the microorganism species[1, 13, 15]. For the analysis of volatiles from food, GC-MS is commonly used and can provide high sensitivity and precision but requires an appropriate sampling process[16, 17]. Various new methods and instruments for sampling volatiles from fruits have been developed[18, 19]. However, these methods require complex protocols and cannot achieve rapid and continuous measurements. Some studies have used E-nose to analyze the volatiles from food and demonstrated that food spoilage can be characterized through chemometrics analyses[17, 20-26], but E-nose is not suitable for on-line measurements since it is complex and costive.

Infrared spectroscopy is considered an effective method for gas measurement[27]. Because it is fast, flexible and provides the ability to measure gases on-line, infrared spectroscopy has been used for qualitative and quantitative measurements of hazardous gases, emissions from volcanic eruptions, greenhouse gases emissions, *etc*[28-33]. Harren *et al.* used infrared laser spectroscopy to measure the ethylene, ethane and methane vaporized from plant leaves[34]. In our previous study, we used Fourier Transform Infrared Spectroscopy (FTIR) to detect the volatiles from grapes during spoilage and found that the concentrations of ethanol and esters increased with storage time[35]. However, the sensitivity of the spectroscopy system was limited due to its short optical path. More recently, we designed a long optical path system consisting of multi-reflecting mirrors to enhance the sensitivity of the system. This system was used to analyze the volatiles from strawberries and successfully identified new volatile compounds[36].

In this study, we observed and analyzed the volatiles from grapes during spoilage by the simultaneous use of long optical path FTIR and sensor arrays comprising carbon dioxide and ethanol sensors. To the best of our knowledge, this is the first study to examine the volatiles from fruits using spectroscopy combined with sensor arrays. The aims of this study were: 1) to observe the characteristic compositions of grape volatiles during spoilage and their changing properties; 2) to demonstrate whether infrared spectroscopy and sensor arrays can be used for grape spoilage discrimination; and 3) to establish a model to classify the grape samples into different spoilage degrees and discuss the influence of the grape quantity.

2. Materials and methods

2.1 Grape samples

The grape samples used in the experiment were of the “Jufeng” variety from

Xiaotangshan (Changping district, Beijing, China). Three groups of 2000 g grape samples that had not been cleaned and pre-treated were placed into 3 identical plastic containers (0.5 m x 0.3 m x 0.3 m). To study the influence of the grape quantity, we also placed 200 g grape samples into identical containers. There were four ventilation holes on the top of each container, each with a diameter of 3 cm. The bottom of the container was connected to a gas cell with a rubber pipe. The experiment was performed at room temperature (22°C). The appearance and aroma of the grapes did not change in the first 3 days, but the grapes developed a soft surface on the 5th day and began to develop obvious mildew on the 7th day.

2.2 Instrumentation

A Vertex 70 FTIR spectrometer (Bruker Ltd., Germany) was used in this study with a liquid-nitrogen-cooled MCT detector. We used an air-cooled ceramic mid-infrared/far-infrared light source. The spectral range was set to 600- 4000 cm^{-1} with a spectral resolution of 0.5 cm^{-1}. A 1 L vacuum air pump, FY-1H (ALUE Ltd., Shenyang, China), was used. A Cyclone™ C2 gas cell (Specac Ltd., UK.) was used. Six reflecting mirrors were used to extend the optical path of the system to 2 m. Our previous study demonstrated that the system sensitivity was significantly enhanced compared with that of a common FTIR spectrometer[36].

The sensor array comprised a carbon dioxide sensor and an ethanol sensor. A COZIR™ Non-Dispersive Infrared Radiation (NDIR) CO_2 sensor (GSS Ltd., UK.) was used with a measuring range of 0- 100% and a precision of 70 ppm. The ethanol sensor used was an electrochemical sensor, C2H5OH-1000 (Membrapor Ltd., Switzerland), with a measurement range of 0-1000 pm and a precision of 20 ppm. We designed the signal processing, control, data collection, display and memory module of the sensor array system, which could record the measurement results automatically. The CO_2 sensor was calibrated before the experiment to reflect the actual concentration of CO_2. We did not calibrate the ethanol sensor because the aim of our study was to determine the relationship between the spoilage process and the output of the sensor without considering the concentration. Moreover, the baseline of the ethanol sensor output was subtracted every 2 hours by placing the sensor in air.

2.3 Spectral measurements

The experiment lasted 8 days. The method of exhausting the volatiles into the gas cell was described in our previous publication. The absorbance spectra were calculated and recorded every 2-3 hours using the spectrum in vacuum as a reference. The sensor array was directly placed into the grape container, and the outputs of the sensors were recorded automatically.

2.4 Spectral data processing

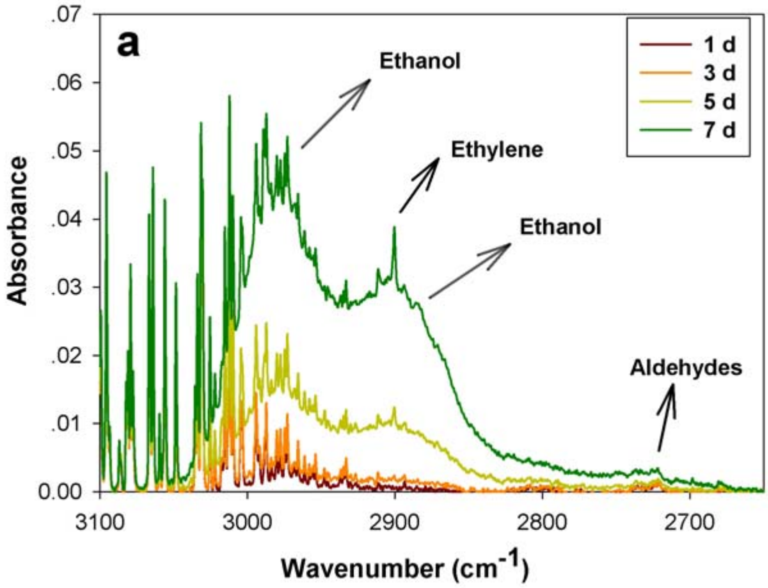
The original spectra were collected using OPUS7.0 software. The pre-processing methods, including baseline correction, low pass filtering and smoothing, were performed in SigmaPlot 12.0. Unscrambler 9.7 was used to perform Principal Component Analysis (PCA) and establish the Soft Independent Modeling of Class Analogy (SIMCA) model.

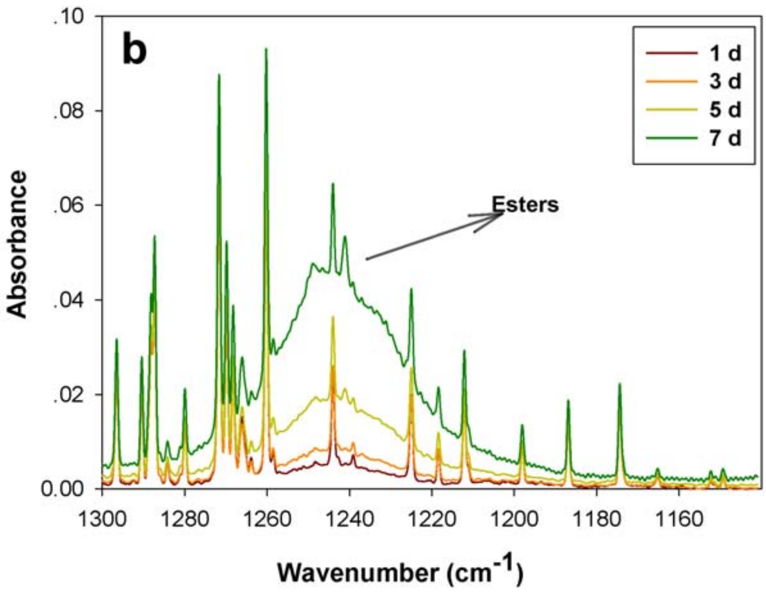
3. Results and discussion

3.1 Spectral characteristics of the volatile compounds

Fig. 1 shows the infrared spectra of the volatiles from the grapes stored in the 1st, 3rd, 5th and 7th day (the grapes were slightly decayed in the 5th day and seriously decayed in the 7th day). The peaks at 3100-2750 cm^{-1}, 1150-950 cm^{-1} and 950-800 cm^{-1} represent the three characteristic bands of ethanol[37], which is vaporized from fruits during spoilage due to anaerobic respiration. It can be observed from Fig. 1(a) and (c) that the decayed grapes vaporized much more ethanol than the fresh ones. The three characteristic bands were very wide, and some absorption peaks from other compounds were overlapped with them. A previous study demonstrated that geraniol is one of the main components of grape aroma[14]. We believe that the 1120-840 cm^{-1} spectral band may be due to geraniol (Fig. 1 (c))[37], which is overlapped with the peaks from ethanol. Linalool is also considered to be present in the aroma of grapes[14], but it is difficult to observe in the spectra of volatiles because it has similar bands to those of ethanol. Esters are the main components in the volatiles of grapes because of the esterification reaction during grape decay[15]. The 1300-1140 cm^{-1} band may be caused by ethyl acetate ($C_4H_8O_2$)[37], which is considered to be the main ester in fruit volatiles[15]. Fig. 1 (b) demonstrates that fresh grapes also release ethyl acetate vapor, but its concentration is much lower than that in decayed grapes. Methyl anthranilate is known to be a special compound in grape volatiles[12]. The 1170-990 cm^{-1} spectral band in Fig. 1 (c) is likely caused by methyl anthranilate, which is overlapped with the wide band of ethanol[37].

In addition to ethanol and esters, aldehydes, ethylene and carbon dioxide are also known to comprise the volatiles from decayed fruits[1]. The 2349 cm^{-1} band is a characteristic band of carbon dioxide but was too strong in our experiment for the long optical path and was not suitable for quantitative analysis (its absorbance was over 0.3). A weaker spectral band, 2285-2170 cm^{-1}, was therefore used to analyze CO_2 (as shown in Fig. 1 (d))[37]. This band contains a wider peak and several narrow peaks, all of which are caused by the absorbance of CO_2. Aldehydes have been demonstrated as a compound in various types of grapes[15]. The spectral peaks at 2830-2600 cm^{-1} may be from aldehydes[37], which are present in both fresh and decayed grapes, with no obvious difference. Ethylene is considered to be a characteristic vapor released from mature fruits[13]. The absorbance peaks at 3010-2950 cm^{-1} are most likely due to ethylene[37], which was present in low quantities in the fresh grapes but in high quantities in the decayed grapes.





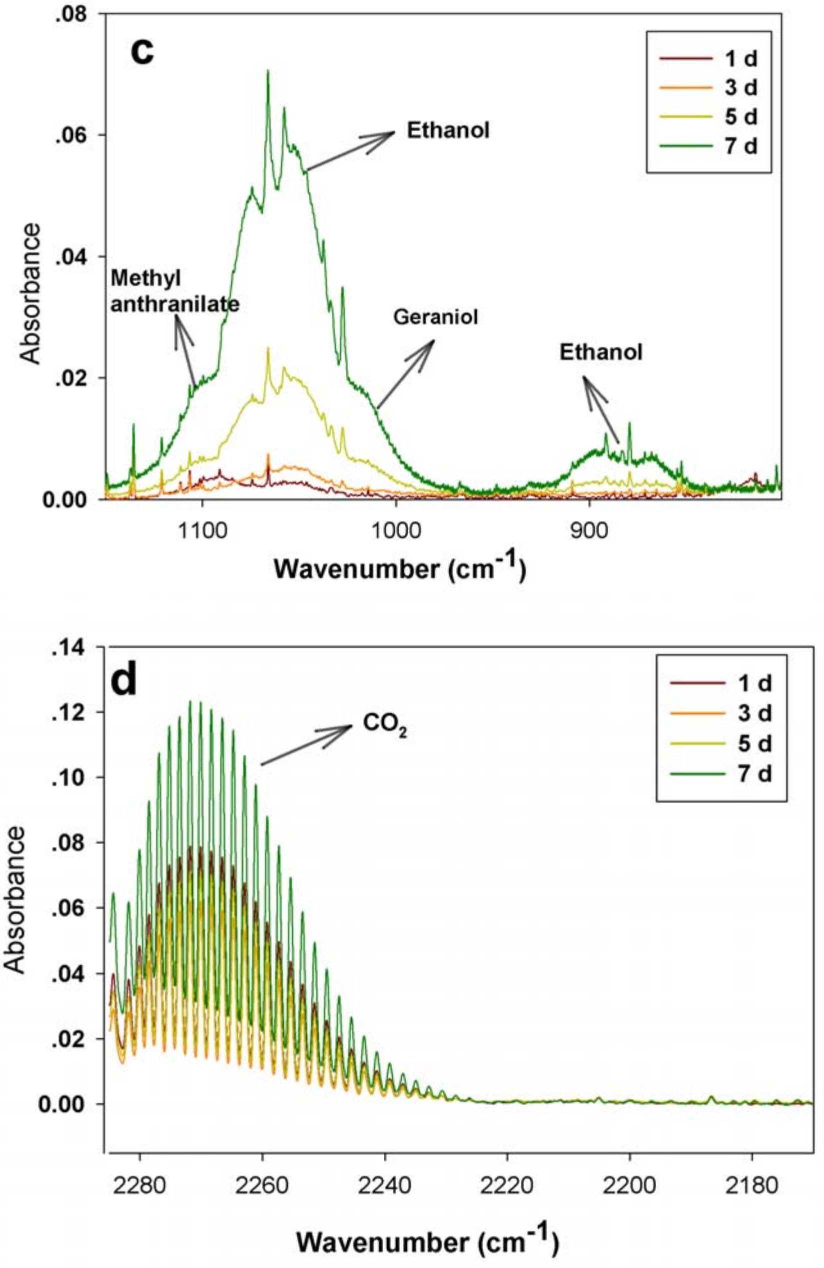


Fig. 1 Spectral characteristics of the volatiles from the grapes stored in the 1st, 3rd, 5th and 7th day

3.2 Changing spectral properties during the spoilage process

The above results demonstrate that there are obvious differences between the spectra of volatiles from fresh and decayed grapes. Furthermore, we also aimed to characterize the spectral changes during the 8-day spoilage process. According to the

Beer–Lambert law, the absorbance intensity is proportional to the concentration for a fixed optical path, so it is possible to calculate the concentration by measuring the heights or areas of the characteristic bands[27]. We used the band area of 1150-950 cm^{-1} to measure the concentration of ethanol and the band area at 1300-1140 cm^{-1} for esters. For the analysis of CO_2 because of its strong absorption, we used the 2285-2170 cm^{-1} band, which includes a low frequency signal overlapping with high frequency signals that complicate analysis. To remove the high frequency signals, we pre-processed the original spectra using low-pass filtering (Fig. 2 (a)) followed by smoothing (Fig. 2 (b)). Then, the spectrum only contained a wide character band that could be used for CO_2 measurement.

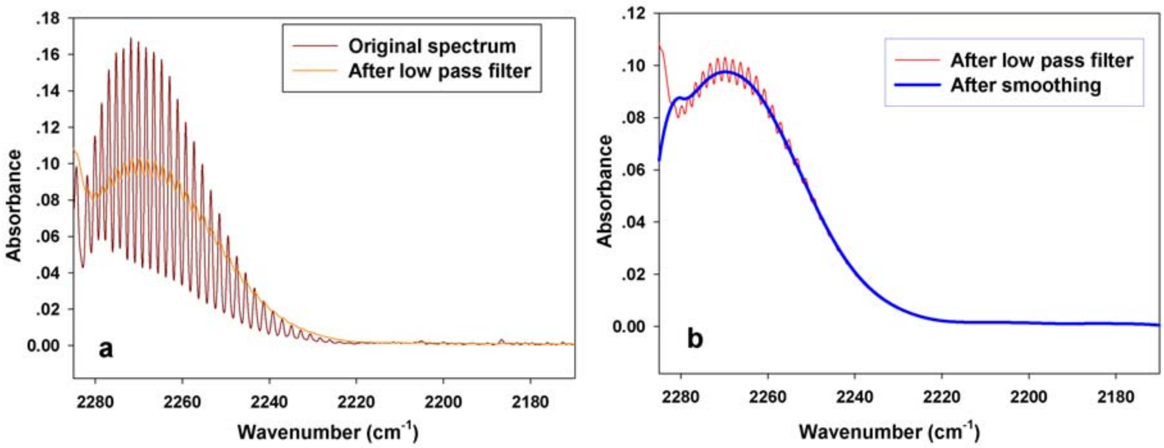


Fig. 2 The pre-processing method to remove the high frequency signals of CO_2 spectral bands

Fig. 3 shows the changing levels of ethanol, esters and CO_2 during grape spoilage calculated by the above analytical methods. It is demonstrated that the concentrations of all three vapors increased with storage time. Compared with the esters, the concentration of ethanol increased more rapidly, especially in the period from 80-140 h, in which the grapes decayed aggressively. Our previous study demonstrated that esters decrease during the serious spoilage period of strawberries[36], but this was not observed in the current study. The concentration of CO_2 also increased with storage but was not obvious in the earlier stage of spoilage. Unlike the changes in ethanol and esters, the changes in CO_2 showed a periodic behavior that may have been caused by microbial respiration. It can also been studied from Fig.3(a) that the curve is nearly flat in the first 1-3 days, then the slopes become positive till the 5th day and then it goes almost flat again. That is consistent with the freshness of the grapes that they were fresh in the 1-3 days, slightly decayed in the 5-6 days and seriously decayed in the 7-8 days.

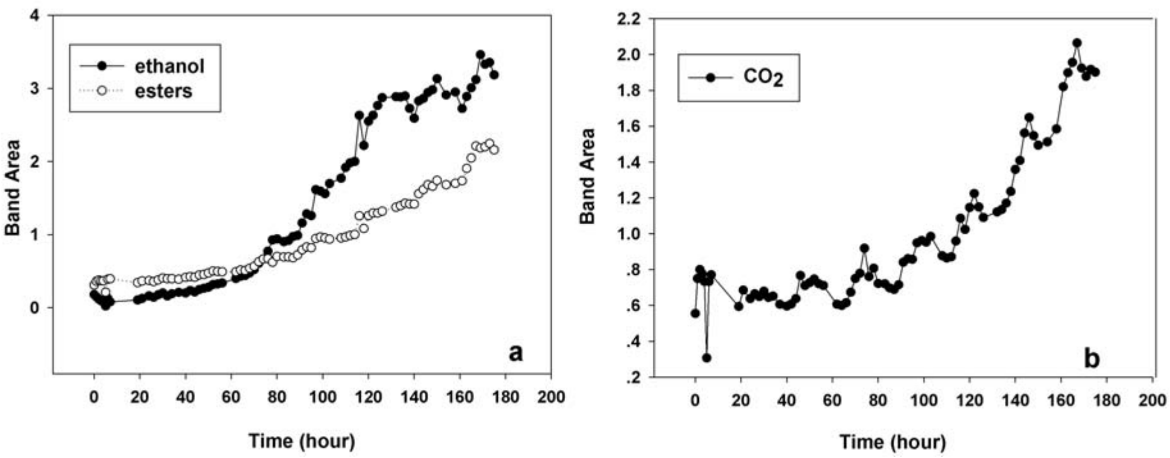


Fig. 3 The changing of ethanol, esters and CO_2 spectral characteristics during grape spoilage

The sensor array was also used to measure changes in volatiles during spoilage, as shown in Fig. 4. The error bars in the figure were calculated from 10 continuous measurements. The changes in the CO_2 concentrations showed similar behavior to that observed by infrared spectroscopy, which also showed a periodic trend. The changes in the output of the ethanol sensor were also similar to the spectroscopy results, but with slight differences that were most likely caused by the low sensitivity of the sensor and the low concentration of the ethanol vapor. The above results demonstrate that both infrared spectroscopy and sensor arrays have the ability to discriminate between fresh and decayed grapes.

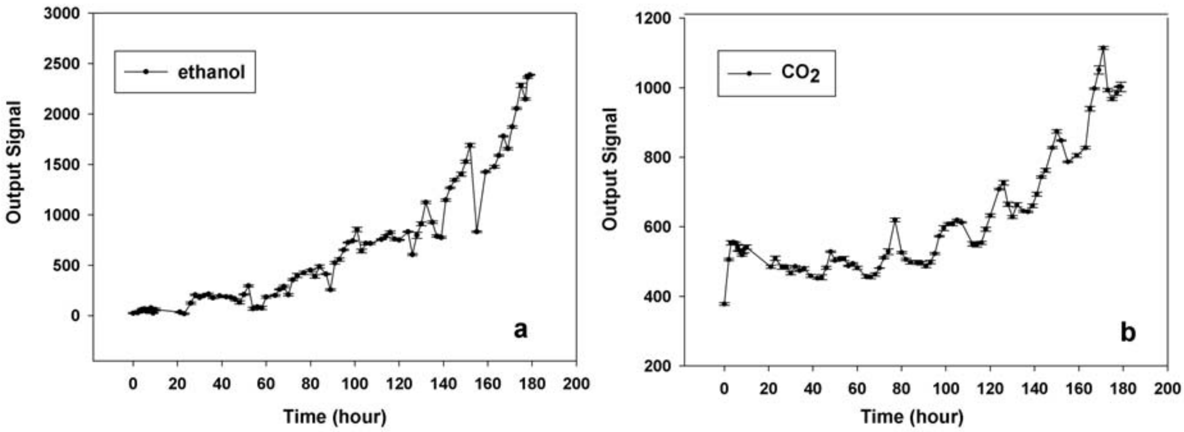


Fig. 4 The changing of the outputs of the sensor array during grape spoilage

3.3 PCA analysis of the infrared spectra of the volatiles from grapes

The above analysis demonstrates that several types of compounds show changing levels during grape spoilage, so PCA analysis was used to reduce the dimension of the data and to allow discrimination of the decayed grapes[38]. In this experiment, the grapes stored for 1-3 d were considered as fresh, while those at 4-6 d were slightly decayed and those at 7-8 d were seriously decayed. The morning of the 4th day and the afternoon of the 6th day were considered transition periods and were not included in the analytical data. PCA analysis was performed using the spectral data in the range of 3100-2750 cm^{-1} and 1300-800 cm^{-1}, which covers the majority of the spectral characteristics of ethanol and esters. The number of Principal Component (PC) was set to 10. Fig. 5 shows the PCA map and demonstrates that the three groups of samples can be easily classified. Several samples were outside the classification areas, perhaps due to small differences in the operations. These results indicate that the volatiles from fresh, slightly decayed and seriously decayed grapes displayed obvious differences in their infrared spectra.

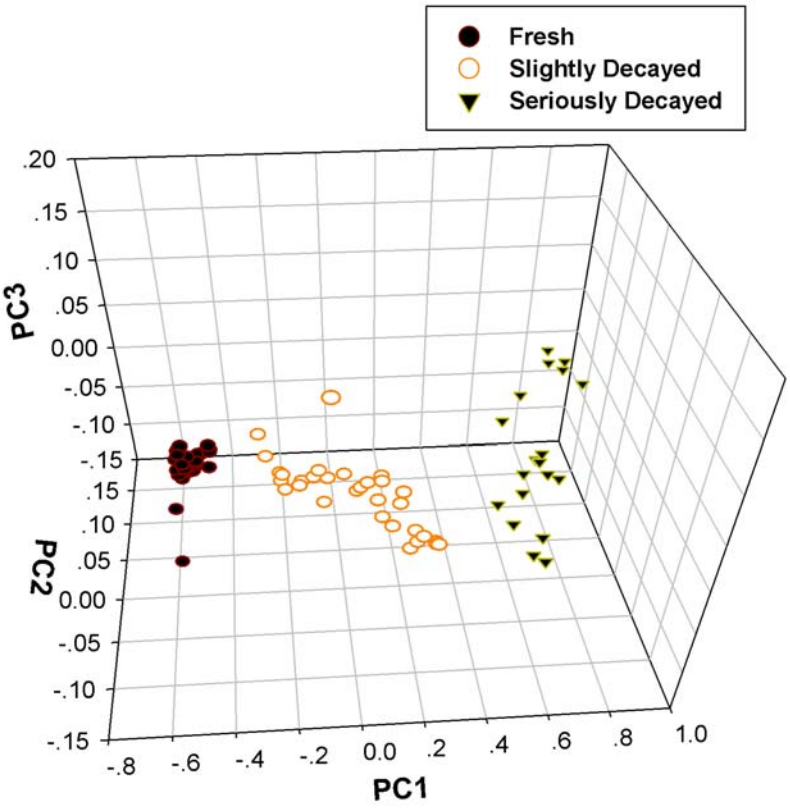


Fig. 5 PCA analytical results for the grapes of different spoilage stages using infrared spectra

We also analyzed the three groups of samples using the output of the sensor array. Fig. 6 shows that most of the samples can be classified, while some points are overlapped. As shown in Fig. 4, the output of the sensor array was somewhat variable for the low concentrations of the volatiles. The sensor array was able to classify the decayed grapes, but its sensitivity was lower than that of infrared spectroscopy. If we were to establish a linear model to attempt to discriminate the three groups of samples, there might be some errors. However, the performance of the model would be much better if only fresh and seriously decayed grapes were analyzed.

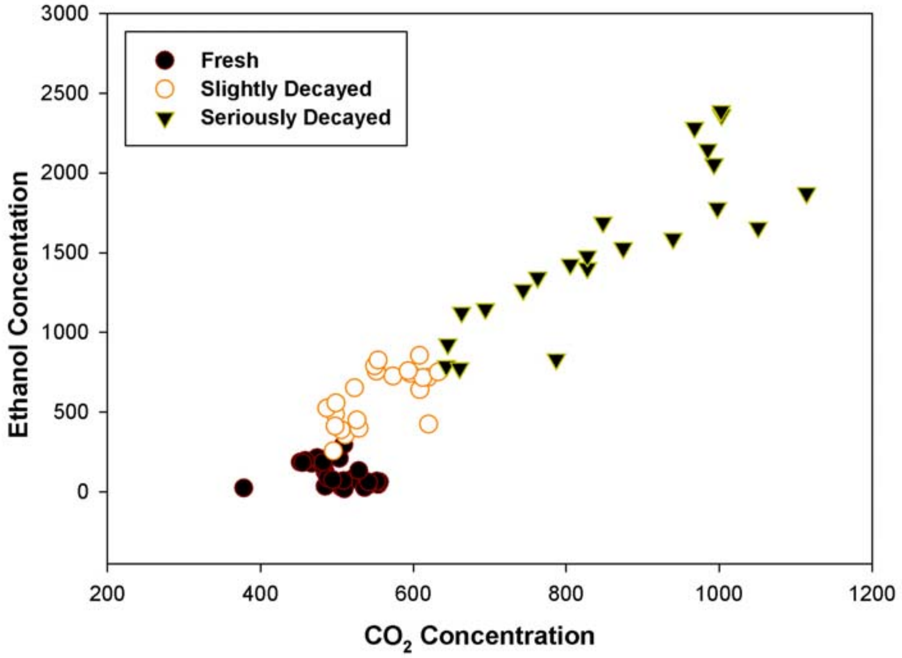


Fig. 6 Analytical results for the grapes of different spoilage stages using sensor array

3.4 SIMCA model for classifying grapes into different spoilage degrees

To further study the ability of infrared spectroscopy to classify grape spoilage, we established a Soft Independent Modeling of Class Analogy (SIMCA) model based on PCA analysis[38]. The correction sets included 10 samples for each group (fresh, slightly decayed and seriously decayed), and each group contained at least one sample for each day. Other samples were included in the prediction sets. As shown in Tab. 1, the correct classification rates for the three groups of samples were all 100%. However, 3 fresh grapes were classified as both fresh and slightly decayed. These 3 samples were from 1500 h, 1800 h and 2100 h on day 3, which are in the transition period between fresh and slightly decayed, suggesting that the volatiles may have already evolved considerably.

Tab.1 The results of SIMCA model to discriminate different spoilage stages of grapes

no. of grape samples	no. of samples classified as fresh grapes	no. of samples classified as slightly	no. of samples classified as seriously	correct classification

			decayed grapes	decayed grapes	
fresh grapes	15	15	3	0	100% [a]
slightly decayed grapes	16	0	16	0	100%
seriously decayed grapes	12	0	0	12	100%

a Three fresh grape samples were classified as both fresh grapes and slightly decayed grapes

Another problem in applying the method is the influence of the quantity of grapes, because the concentrations of volatiles can vary with the quantity of the sample. We attempted to use a quantity standardization method to pre-treat the spectra before classification. As described in the experimental section, we used 200 g samples to study the influence of the grape quantity. From a quantity point of view, the volatiles from the 2000 g sample should be 10 times greater than those from the 200 g samples. However, by comparing the spectra of the two quantities of grapes on the same day, we found that the multiplication factor was approximately 8, which may be due to the interrelationship between each grape. We multiplied the spectra from the 200 g sample by 8 and mixed them into the spectra from the 2000 g samples. Six 200 g samples were recorded for each spoilage stage; 2 samples were added into the correction sets and 4 samples were added into the prediction sets. Tab. 2 shows the results of the new SIMCA model, which demonstrates that the classifications of the 2000 g samples were not altered significantly compared with the classifications of the previous SIMCA model by adding a sample that was classified as both fresh and slightly decayed. For the 200 g samples, the fresh and seriously decayed groups could be classified easily (with correct rates of 100% and 75%), while the slightly decayed grapes were difficult to discriminate. This result indicates that the SIMCA model combined with quantity standardization is effective for the classification of fresh and seriously decayed grapes. We also used the sensor array to measure the volatiles from the 200 g samples, but the output of the sensor was not stable enough for the low concentrations of volatiles from the 200 g sample.

Tab.2 The results of SIMCA model considering the influence of the quantities of grapes

	no. of all grape samples (no. of 200 g samples)	no. of all samples (no. of 200 g samples) classified as fresh	no. of all samples (no. of 200 g samples) classified as slightly decayed	no. of all samples (no. of 200 g samples) classified as seriously decayed	correct classification for all samples (200 g samples)
fresh grapes	19 (4)	19 (4)	4 (0)	0	100% [a] (100%)
slightly decayed grapes	20 (4)	0	17 (1)	0	85% (25%)
seriously decayed grapes	16 (4)	0	0	15 (3)	93.8% (75%)

a Four fresh grape samples were classified as both fresh grapes and slightly decayed grapes

4. Conclusions

This study demonstrated that grapes release specific volatiles during spoilage and that the presence and concentrations of these volatiles changes with the spoilage degree. Infrared spectroscopy was verified to be an effective tool for the discrimination of grape spoilage stages based on their volatiles, with the advantages of being fast, requiring no contact and allowing continuous measurement. To create a tabletop instrument with a long fixed optical path, we exhausted the volatiles into a gas cell for measurement. In on-line applications, it is possible to obtain a long optical path by putting the light source and the spectrometer on the opposite side of a storehouse[32]. Because the aim of this experiment was to demonstrate the ability of infrared spectroscopy to classify grape spoilage degrees, a wide band FTIR spectrometer is used. For practical use, simple and low cost systems, such as tunable diode laser absorption spectroscopy (TDLAS) systems, can be considered[39, 40].

We also studied the ability of sensor arrays to identify decayed grapes, which can be considered a simplified form of infrared spectroscopy. Although the outputs of the sensor arrays were not very stable, they still provided the ability to discriminate fresh and decayed grapes. A number of problems, such as the stability and baseline drift of the sensor, should be considered in field measurements, especially when the quantities of samples are low. We subtracted the baseline of the ethanol sensor every 2 h, but this correction is too complex for use in real-world applications. We believe that an effective way to enhance the performance of the sensor array is to use infrared sensors instead of electrochemical sensors[41], which is similar to a spectroscopy method.

We also investigated the use of the classification model for different quantities of grapes but did not achieve ideal results. Because of the interrelationship between each grape, the quantity standardization method was not effective. A more effective method may be to design a more flexible quantity compensation method according to the vapor properties of different quantities of grapes. Another method would be to use concentration changes as the variables instead of the absolute values of the concentrations and then to establish new classification models.

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