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Non-intrusive prediction of fruit spoilage and storage time *via* detecting volatiles in sealed packaging using laser spectroscopy

Yuan Gao [a,b], Leizi Jiao [b], Fu Jiao [b], Daming Dong [a,b,*]

[a] *School of Electronic Engineering and Automation, Guilin University of Electronic Technology, Guilin, 541004, China*
[b] *National Engineering Laboratory for Agri-product Quality Traceability, Beijing Research Center for Intelligent Equipment for Agriculture, Beijing Academy of Agriculture and Forestry Sciences, Beijing, 100097, China*

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ABSTRACT

Monitoring fruit spoilage can effectively avoid waste and reduce economic losses. Recent studies have proved that the measurement of food volatile compounds is effective to detect food spoilage at its early stage. Indeed, some fast analytical methods have been developed to measure the volatiles released from food, such as proton transfer reaction mass spectrometry, gas-phase infrared spectroscopy, and E-nose. However, these methods can only be applied to measure volatiles in unsealed packages of food and are unable to detect volatiles that are sealed in containers or packages, which are the common situations in food storage and sales. Herein, we investigated a non-intrusive method for detecting food volatiles and monitoring fruit spoilage during storage. A laser directed incident radiation into the food package where it diffused through the box, and absorption spectra were generated. The fingerprint spectra of the molecules in food volatiles were used to determine the concentrations of the volatiles inside the food package. In the experiment, grape spoilage was monitored by measuring changes in oxygen concentration and we successfully predicted the beginning of grape spoilage on the seventh day. The root-mean-square errors of prediction for the storage times of grapes and strawberries were 0.4426 and 0.3079 days, respectively.

1. Introduction

Fruit is popular worldwide as a nutritious food with a wide range of flavors and textures. However, fruit requires complex growing and ripening conditions and often transportation over long distances, which allows the fruit to decay easily, causing heavy economic losses and food safety concerns (Hui et al., 2010). Therefore, there is an urgent need to develop a rapid and non-intrusive detection method for monitoring fruit in real time during storage and transportation.

Volatile compounds from fruit and other plants can reflect their particular status because they are associated with plant disease (Li et al., 2019) and self-protection (Dudareva, Negre, Nagegowda, & Orlova, 2006), the freshness or maturity of the plants (Dong, Jiao, Li, & Zhao, 2019), and their nutritional composition (Goff & Klee, 2006). Studies have reported that the quality of fruit can be monitored based on these volatile compounds, thus enabling their storage time to be extended (Lanciotti et al., 2004). Early studies on fruit volatiles have usually used gas chromatography–mass spectrometry and other mass spectrometry methods to analyze the specific volatiles related to the physical and chemical properties of fruits. This approach has often been combined with chemometrics to detect the onset of fruit spoilage or for classification (Diez-Simon, Mumm, & Hall, 2019; Fernando, Ramarathnam, Krishnamoorthy, & Savchuk, 2005; Lubes & Goodarzi, 2017). However, sample pretreatment or combination with other methods has often been necessary to detect volatiles, making the process complex and costly. Some studies have used an E-nose to determine the stages of fruit spoilage and thus predict the fruit storage time (Hui, Wu, Ye, & Ding, 2013; Jia, Liang, Jiang, & Wang, 2019; Liu, Liu, Xu, Guan, & Wu, 2019; Song et al., 2019). The E-nose allows online measurements to be made using small sample volumes at low cost but its sensors only have a short useful life and suffer from cross-sensitivity in practical applications, so are not suitable for making measurements in real time. In addition, photoacoustic spectroscopy has been developed in the fruit industry recently and shows great prospects for application because of its high sensitivity and repeatability (Popa, 2019).

Infrared and laser spectroscopy are techniques recently used to analyze fruit volatiles. Infrared spectroscopy has been widely used in gas analysis because of its rapid detection and high sensitivity to different

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* Corresponding author. School of Electronic Engineering and Automation, Guilin University of Electronic Technology, Guilin, 541004, China.
E-mail addresses: damingdong@hotmail.com, dongdm@nercita.org.cn (D. Dong).

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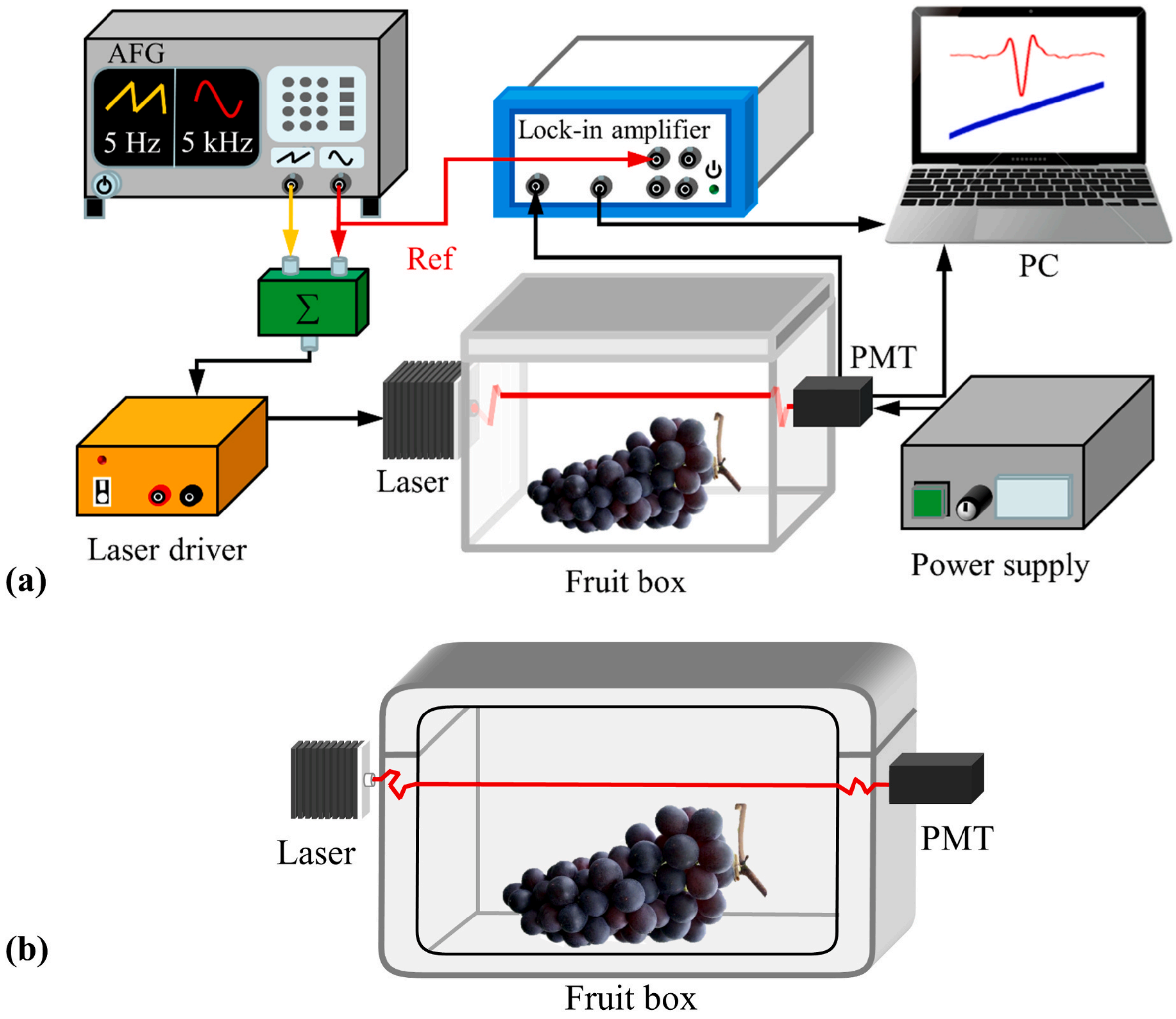

Fig. 1. Schematic diagrams of system used for detection for oxygen in sealed fruit boxes by tunable diode laser spectroscopy: (a) experimental arrangement used for detection; (b) longitudinal section diagram of fruit boxes used in the experiments.

molecules. These advantages help measure the volatiles and detect fruit quality rapidly. Volatiles from fruit, such as grapes, strawberries, and mangoes, have been measured using a long-path Fourier transform infrared spectrometer (FTIR) and a thermal infrared camera with a band-pass filter for predicting the stages of fruit spoilage (Ding, Dong, Jiao, & Zheng, 2017; Dong et al., 2013, 2014; Jiao, Dong, Han, Zhao, & Du, 2017; Jiao, Guo, Chen, Zhao, & Dong, 2019). These methods require almost no pretreatment and greatly improve the detection efficiency, thus providing a new direction for analyzing fruit volatiles. However, the application of this approach is limited because FTIR requires the extraction of the fruit volatiles into a gas cell and the thermal infrared camera with a band-pass filter cannot detect several gases simultaneously. An obvious problem is also that neither infrared nor mass spectrometry techniques can directly measure the gas in sealed packaging. In a practical situation, the method of detection cannot be allowed to damage the packaging. Therefore, a method that could monitor packaged fruit without the need for gas collection or opening the package would further simplify the detection process and improve its efficiency, and so be of greater practical significance.

Gas in scattering media absorption spectroscopy (GASMAS), proposed by Sjöholm, Somesfalean, Alnis, Andersson-Engels, and Svanberg (2001), presents a solution to monitoring volatiles in sealed packaging. GASMAS is a laser technology that can analyze the gas in scattering media. GASMAS has been used to analyze gas in the human sinus cavity (Larsson et al., 2019; Svanberg et al., 2016; Zhang, Huang, Li, Svanberg, & Svanberg, 2015), in food and food packaging (Lewander, Guan, Persson, Olsson, & Svanberg, 2008; Li, Lin, Zhang, Svanberg, & Svanberg, 2017; Li, Li, Hu, Svanberg, & Svanberg, 2019), in fruit respiration and gas exchange (Huang et al., 2016; Joseph, Van Beers, Postelmans, Nicolai, & Saeys, 2021), in the pore structures of materials (Svensson, Adolfsson, Lewander, Xu, & Svanberg, 2011), in pharmaceuticals (Svensson et al., 2008), and in other fields. GASMAS has excellent application prospects and development potential because scattering media, such as clouds, fog, fruit, foam, paper, and human tissue, are ubiquitous in daily life. Fruit packaging is usually made of scattering media such as foam or thick paper. A laser can be emitted into the fruit package directly, and the absorption spectra can be resolved from the background spectra because the free gas exhibits absorptive imprints that are typically 10,000 times narrower than those of solid-state materials (Svanberg, 2013).

In this study, we proposed an innovative approach to predict the fruit storage time and detect fruit quality by measuring the oxygen concentration in the headspace of sealed fruit packaging without damaging the packaging by combining the use of GASMAS and tunable diode laser spectroscopy (TDLS). The major advantages and innovations of our method are that it does not require sample pretreatment and can monitor gas in sealed packaging non-intrusively, which allows it to be used in practical situations in food storage and sales. To the best of our knowledge, this study is the first to detect fruit spoilage without damaging the packaging by measuring fruit volatiles inside the sealed

Fig. 2. Photographs of the experimental sample and set-up: (a) fruit boxes before being sealed; (b) sealed fruit box under study, with the PMT placed on one side of the box and the laser directed from the other side.

packaging.

2. Materials and methods

2.1. Materials

The fresh grapes and strawberries used in the experiment were picked when mature. The strawberry samples came from Xiaotangshan in the Changping District (Beijing, China) and the grape samples from the Yanqing District (Beijing, China). The grapes were cut at the main stem, leaving the top side stem attached to each grape. This allowed more grapes to be packed into the foam box while protecting the grapes with the side stems. The grapes and strawberries were packed in food-grade foam boxes with dimensions of 24 × 14.5 × 18.5 cm and a wall thickness of 1.5 cm. Both types of fruit were not washed. The foam boxes were filled as much as possible, leaving a headspace to avoid any backscattering of light from the fruit then sealed by the lid.

2.2. Experimental set-up

A schematic diagram of the experimental system is shown in Fig. 1. A continuous-wave distributed feedback (CW-DFB) laser (Nanoplus, Nanosystems and Technologies, Gerbrunn, Germany) operating at 760 nm was used as the light source to monitor the oxygen absorption lines. The power of the laser was 5 mW. A laser diode driver (WLD3343, Wavelength Electronics, Bozeman, MT, USA) and a temperature controller (WTC3243, Wavelength Electronics) were connected to the laser. The laser wavelength scanning was performed by changing the current of the DFB laser under constant temperature conditions. The laser was operated at a temperature of 25 °C. A ramp wave of 5 Hz was used to ensure that the laser output wavelength passed through the oxygen absorption lines. Wavelength modulation technology was also used to improve the signal-to-noise ratio because of the very weak intensity of the scattered light (Mei & Svanberg, 2015). A sinusoidal wave with a frequency of 5000 Hz was injected into the laser driver for modulation. The modulation depth was adjusted to obtain the optimal signal-to-noise ratio. The ramp and sinusoidal waves were output using an arbitrary function generator (AFG31000, Tektronix, Beaverton, OR,

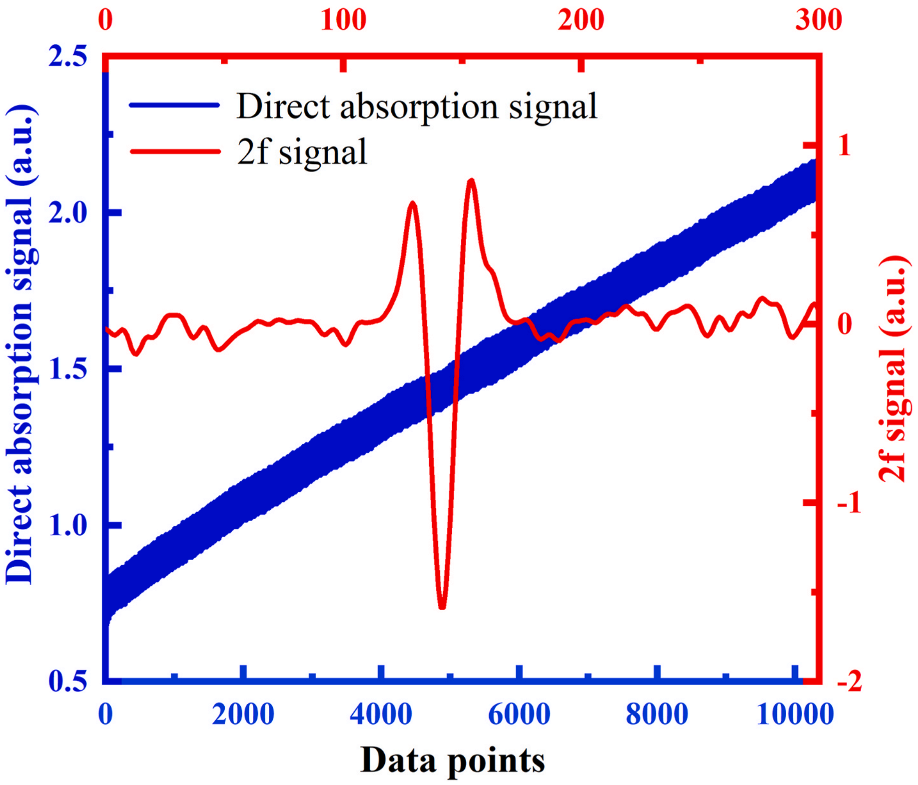

Fig. 3. Typical direct absorption and 2f signals in a period.

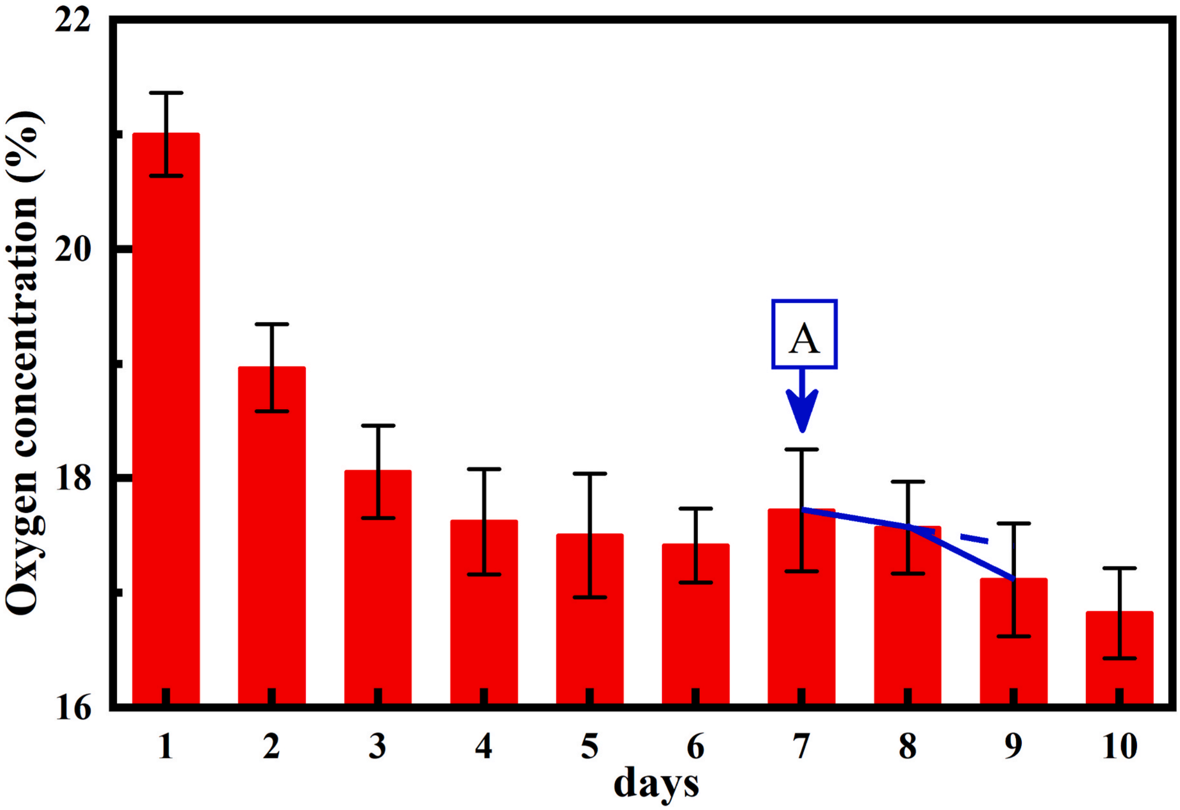

Fig. 4. The oxygen concentration in the grape box was measured for ten consecutive days. A: the beginning of the increase in oxygen consumption, which can be regarded as an indicator of grape spoilage.

USA) with four channels. The ramp and sinusoidal waves were coupled into the laser by an adder. The laser was tightly attached to the foam box and the scattered light passing through the box was monitored from the other side. The foam, as a typical scattering media, had large particles and pores. The light was scattered inside the foam and therefore through the foam. Fig. 1b shows a longitudinal section drawing of a foam box to illustrate the path of light transmission. Initially, a conventional silicon photodiode was used as the detector, but could not monitor the oxygen level because of its low sensitivity. Therefore, a photomultiplier tube (PMT) with a higher sensitivity was used as the system detector. A PMT (H10722-20, Hamamatsu Photonics, Hamamatsu, Japan) with adjustable gain was used as the detector to monitor the scattered light. A lock-in amplifier (MFLI, Zurich Instruments, Zurich, Switzerland) was used to demodulate the signal and output harmonic signals. Data were acquired using the built-in MFLI software.

2.3. Methods

The boxes of fruit were stored at 20 °C. Photographs of the fruit before sealing and during the experiment are shown in Fig. 2.

The light absorption of gases obeys the Beer-Lambert law (Larkin, 2017) expressed as:

$$A = \ln(\frac{1}{T}) = \ln(\frac{I_0}{I}) = aCl \qquad (1)$$

where A is absorbance, T is transmittance, C is concentration, l is path length, I_0 is incident intensity, and I is transmitted intensity. According to the Beer-Lambert law, the absorbance, A, is proportional to the gas concentration, C, and the optical path, l. The detection sensitivity can be improved by enhancing the optical path. Therefore, the longer side of the foam box was used as the detection path in the experiments. The sensitivity of the signal to the position was not significant during the experiment because the direction of the transmitted light after passing through the scattering media was uncertain. In our study, the laser and detector were set up in a straight line to maximize the stability of the system.

The grapes and strawberries were measured for 10 and 5 consecutive days, respectively, after the boxes were sealed. For the monitoring of grape spoilage, two control groups were also stored in the same environment to allow a comparison of the status of the grapes on the corresponding days of storage. The measurements were made at the same time each day, with the second harmonic (2f) and direct absorption signals being acquired simultaneously. Typical 2f and direct absorption signals during a period are shown in Fig. 3. The direct absorption signal, S_{Dir}, was fitted to obtain the signal intensity without absorption at the absorption line center. The peak-to-peak value of the 2f signal, $S_{2\mathrm{f}}$, was calculated as the effective harmonic signal. To normalize the signal, the final signal, S, can be expressed as:

$$S = \frac{S_{\mathrm{Dir}}}{S_{2\mathrm{f}}} \qquad (2)$$

This method is not affected by laser fluctuation or PMT gain, so the signal is only related to the oxygen concentration. The PMT gain can also be adjusted during measurement to obtain the maximum absorption without saturation, and the sensitivity can be improved when a weak absorption is measured. To reduce the system noise, 100 periodic signals were averaged to provide the final value.

3. Results and discussion

3.1. Quantification of oxygen concentration in the foam box

According to the Beer-Lambert law, the gas concentration cannot be calculated without measuring the path length. However, the path length in the foam box is unknown so it is impossible to quantify the oxygen concentration directly. Instead, the equivalent mean path length (L_{eq})

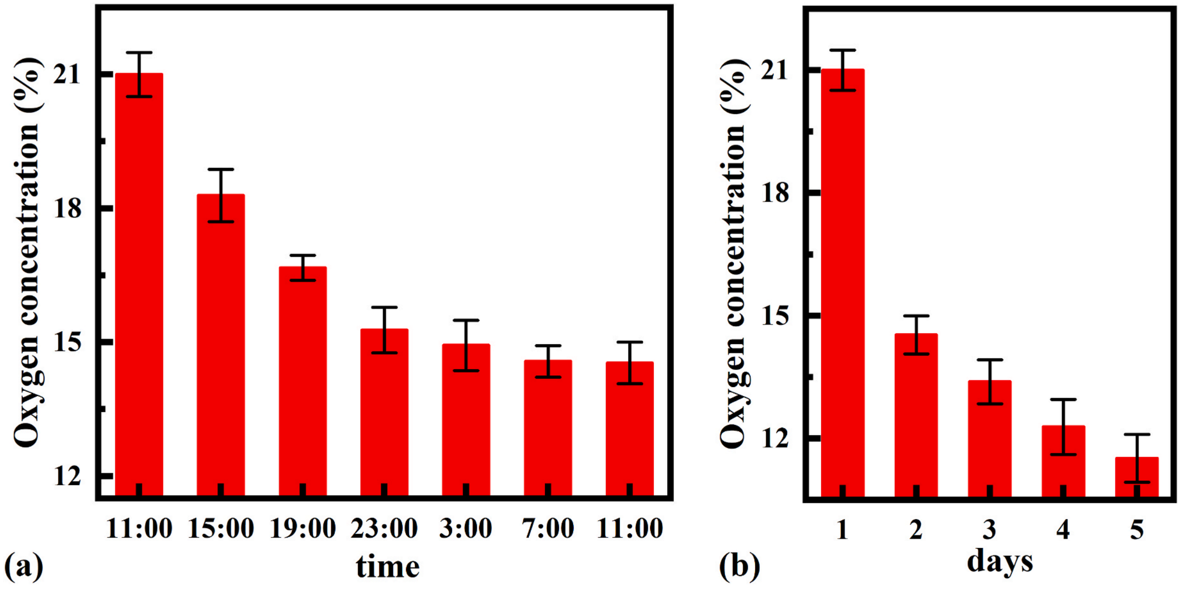

Fig. 5. The oxygen concentration in the strawberry fruit box was measured for 5 days: (a) measurements at 4-h intervals on the first day; (b) measurements for five consecutive days.

was used, which is the path length that the light passes through in air with a known gas concentration as a reference, which exhibits the same gas absorptive imprint in the sample (Li et al., 2017). An advantage of this method is that a standard gas calibration is not required. Moreover, a polynomial was introduced into the fitting process to eliminate the effects of signal drift and other errors.

It is noted that L_{eq} can be regarded as a quantitative result. To display the result more intuitively, the sample oxygen concentration, $C_{sm}(1)$, can be calculated using L_{eq} as follows:

$$C_{sm}(1) = C_{sm}(2) \times \frac{L_{eq}(1)}{L_{eq}(2)} \tag{3}$$

where $L_{eq}(1)$ and $C_{sm}(1)$ are the equivalent mean path length and oxygen concentration of the sample, respectively; and $L_{eq}(2)$ and $C_{sm}(2)$ are the equivalent mean path length and oxygen concentration of the reference gas (atmosphere), respectively. In this experiment, the oxygen concentration, $C_{sm}(1)$, can be calculated because the oxygen concentration in the freshly sealed foam box is equal to the oxygen concentration in the atmosphere (21%).

3.2. Change in oxygen concentration in packages during fruit storage and spoilage

The oxygen concentration in the boxes of grapes and strawberries was measured at the same time each day. For the boxes of strawberries, with their high aerobic respiration rate, the oxygen concentration was also measured every 4 h on the first day, thus allowing the fruit storage time to be predicted more accurately using this short time interval.

From observing the overall trends, the oxygen concentration in the boxes of both types of fruit decreased significantly during storage because aerobic respiration consumed the oxygen in the boxes. The porous foam material of the boxes also had a level of air permeability so that gas would have been exchanged slowly between the interior and exterior of the boxes. Therefore, the oxygen concentration declined when the respiration rate was greater than the rate of gas exchange through the walls of the boxes. One important reason for the increase in oxygen concentration on some days was that the oxygen consumption rate of the fruit had been less than the oxygen exchange rate through the walls of the boxes.

Table 1
Predicted results of grape spoilage stage with storage time.

Grape status	Fresh	Slight spoilage
Predicted value	1–6 d	7–10 d

Fig. 4 shows the changes in oxygen concentration in the boxes of grapes. The oxygen concentration decreased rapidly during the first 3 days of storage, indicating that the grapes were still fresh with a high rate of aerobic respiration. This trend then changed significantly because of the effects of both the decrease in oxygen concentration and the increasing degree of spoilage from the fourth day. There was a brief increase in the oxygen concentration on the seventh day of storage, possibly because the rate of aerobic respiration had become less than the gas exchange rate through the box walls, causing the oxygen concentration to rise. The oxygen concentration dropped again after the seventh day, indicating an increase in oxygen consumption. This result can be explained in two ways. First, the grapes had begun to decay slightly from the seventh day, so some grape flesh had lost the protection provided by the skin, thus increasing contact with the air and accelerating oxidation reactions. A second explanation is that the number of aerobic microorganisms had increased during storage and consumed the oxygen in the box. This implies that the day when the oxygen consumption rises indicates the onset of grape spoilage.

Fig. 5 shows the changes in oxygen concentration in the boxes of strawberries. The oxygen concentration decreased sharply over the first 2 days. The strawberries were still fresh, so the rate of aerobic respiration was rapid without the protection offered by a thick skin, as observed for the grapes. The oxygen concentration then declined slowly, which was also affected by the freshness of the fruit and the oxygen concentration in the box. A shorter experimental measurement interval of 4 h was thus used during the first day to monitor the faster changes in the respiration rate of the strawberries in more detail, and to allow the storage time to be predicted more accurately (Fig. 5b).

3.3. Analysis of fruit spoilage and shelf-life based on oxygen concentration

When grapes decay, the oxygen consumption should not increase continuously because the rate of aerobic respiration of grapes normally decreases as the grapes spoil. In the boxes of grapes, the spoilage and the increase in the numbers of aerobic microorganisms increased the oxygen

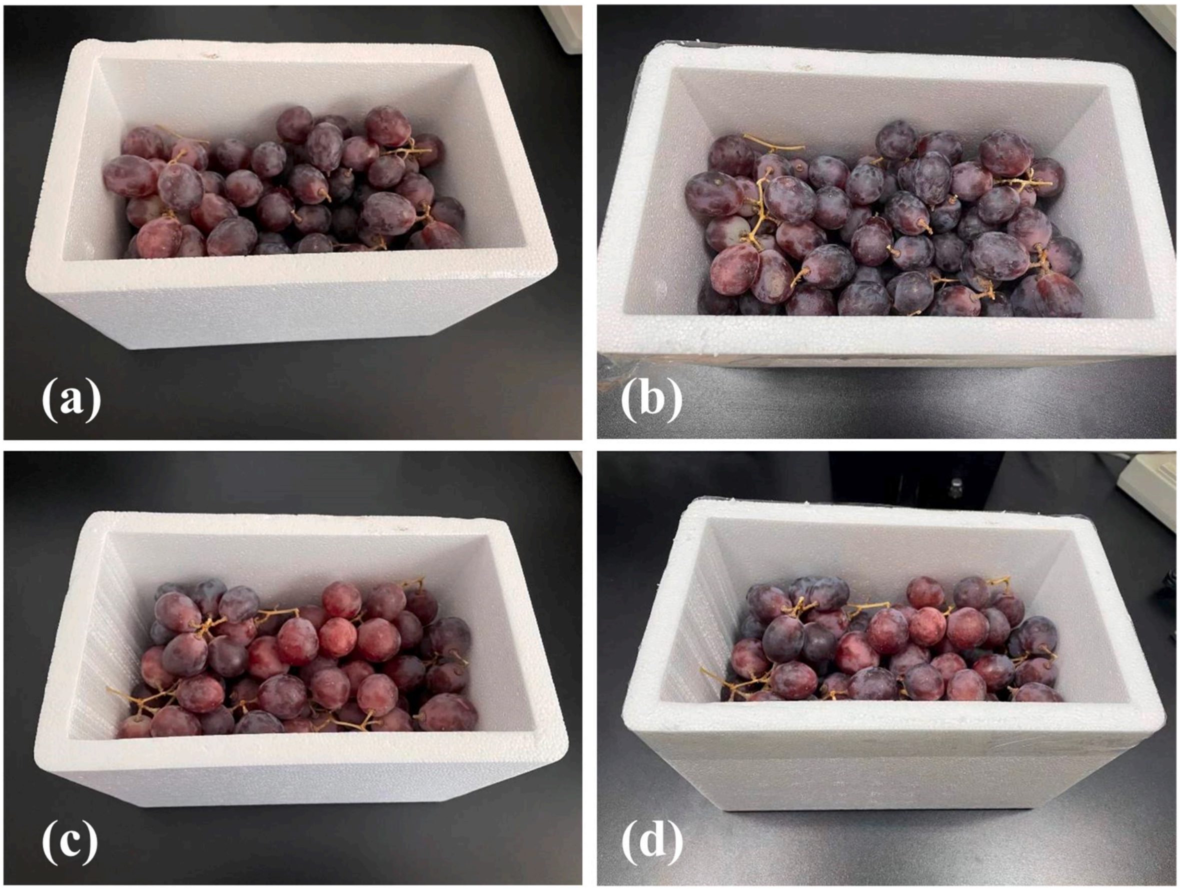

Fig. 6. Photographs of grape samples in two control groups: (a) control group 1 before being sealed; (b) control group 1 was opened on the seventh day; (c) control group 2 before being sealed; (d) control group 2 was opened on the tenth day.

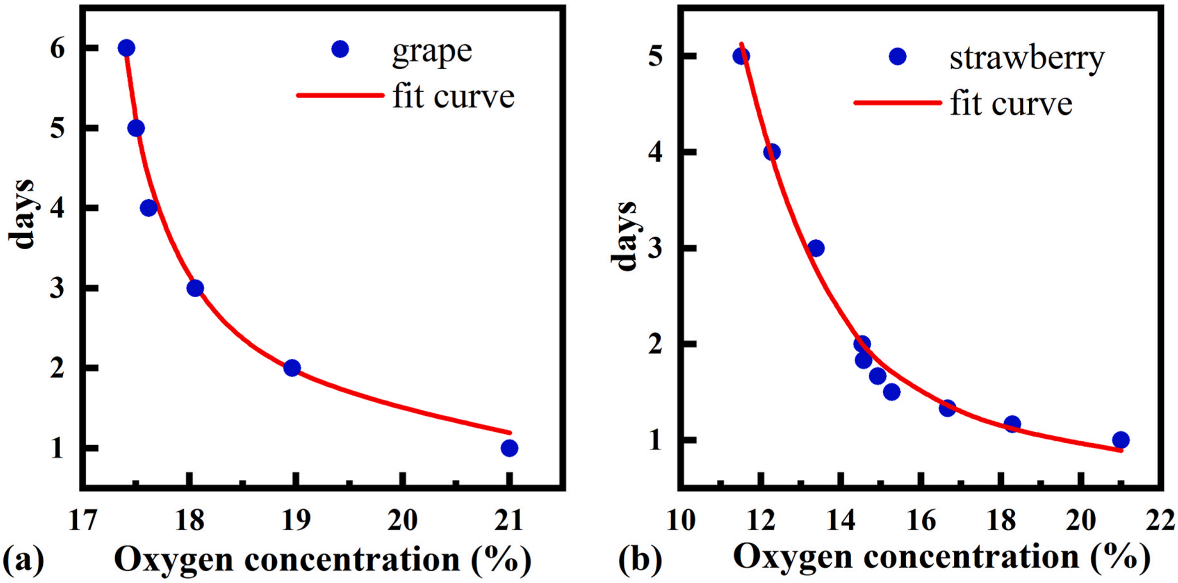

Fig. 7. Regression model of oxygen concentration and storage days: (a) model for grape; (b) model for strawberry.

consumption. Thus, the slope of the line between two consecutive days could be regarded as indicative of grape spoilage. This stage of grape spoilage was predicted based on the explanation shown in Table 1. Two control groups were also stored in the same environment to allow a comparison of the status of the grapes on the corresponding days of storage with that predicted by the measurements of oxygen consumption. When control group 1 was opened on the seventh day, slight alcohol and aromatic odors were apparent and the grapes had become soft and slightly spoiled. The grapes in control group 2, opened on the tenth day, were not very different from those in control group 1, but were softer and slightly spoiled (Fig. 6). Therefore, the control groups indicated slight spoilage on the seventh day of storage, which

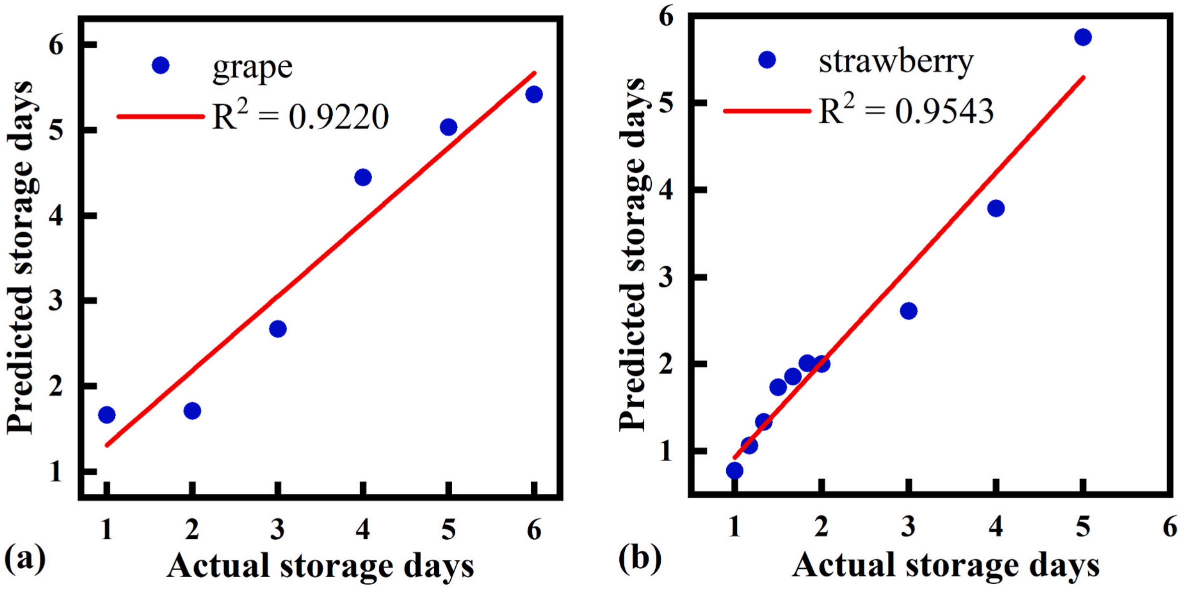

Fig. 8. Predicted results of grape and strawberry samples by leave-one-out cross-validation: (a) grape; (b) strawberry.

Table 2
Comprehensive results of predicting grape and strawberry storage time.

	Calibration		Cross-validation		Prediction	
	R^2_c	RMSEC	R^2_{cv}	RMSECV	R^2_p	RMSEP
grape	0.9892	0.2507	0.9836	0.2332	0.9220	0.4426
strawberry	0.9810	0.1830	0.9808	0.1802	0.9543	0.3079

had been predicted non-intrusively by the proposed experimental method.

According to the relationship between the storage time and oxygen concentration, the storage time of grapes and strawberries in sealed packaging can be predicted by establishing a model of how oxygen concentration varies with storage time. The experimental data from the grapes and strawberries were fitted and regression models were established (Fig. 7). The leave-one-out cross-validation was used to verify the reliability of the regression model (Fig. 8) because leave-one-out cross-validation is suitable for small sample volumes. A comprehensive comparison of the grape and strawberry storage time prediction models is shown in Table 2.

The results show that the interaction between oxygen and fruit is fairly complicated, making it difficult to accurately evaluate the status of the fruit by only measuring the oxygen concentration. However, through continuous measurement of oxygen concentration, it is still possible to predict the storage time of fruit during its fresh stage and to provide a rough estimate of its stage of spoilage when sealed in packaging.

4. Conclusions

In this study, we developed a novel detection method based on TDLS to non-intrusively monitor the gas in sealed boxes of fruit. We measured the oxygen concentration in sealed foam boxes of grapes and strawberries using a DFB laser operated at 760 nm. The results showed that the oxygen concentration in the boxes varied over time during fruit storage. Based on the changes in the oxygen concentration, we established models to predict fruit spoilage and storage time. The current study indicates that laser spectroscopy can be used as a completely non-intrusive method to detect the quality of fruit in sealed packaging, which provides a new direction for the future application of laser spectroscopy. As well as oxygen, other volatile compounds formed in the boxes of fruit, such as ethanol and ethylene, can be monitored using this method. Such analysis would allow the stages of spoilage and storage times of packaged fruit to be accurately predicted in detail, therefore providing meaningful monitoring of fruit quality in practical situations.

CRediT authorship contribution statement

Yuan Gao: developed the sensor and performed the measurements, analyzed the data. and. **Leizi Jiao:** developed the sensor and performed the measurements, and, analyzed the data. All authors contributed to the data interpretation and writing of the manuscript. **Fu Jiao:** developed the sensor and performed the measurements. **Daming Dong:** conceived and supervised the project.

Declaration of competing interest

The authors have no competing interests to declare.

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