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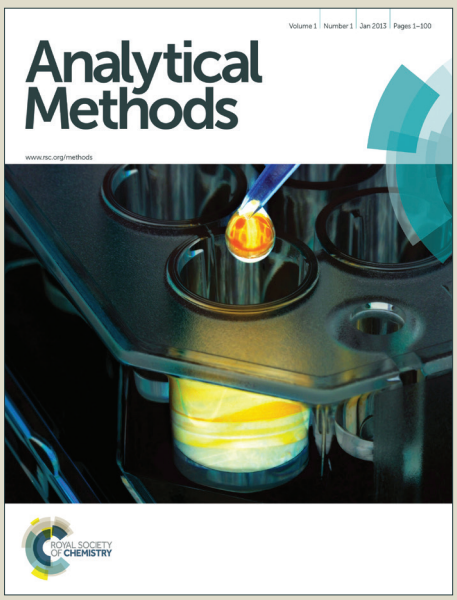

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Full Papers

Identification of Beef Spoilage *via* the Analysis of Volatiles Using Long Optical -path Fourier Transform Infrared Spectroscopy

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Based on the analysis of specific volatile substances released during beef spoilage, the beef status can be predicted. During beef spoilage, ammonia, carbon dioxide, alcohols, esters, aldehydes, ketones, and other substances are released and can be detected by the long-path FITR (Fourier transform infrared spectroscopy). The concentrations of these substances vary with storage time. We could distinguish beef in different stages of spoilage using FTIR spectrometry combined with chemometric methods. The study indicated that infrared spectroscopy could be used to monitor the spoilage status of beef.

1 Introduction

Beef is one of the important foods in the world.[1] In order to guarantee safe supply, it is necessary to develop a method to real-time monitor and supervise the quality status of beef because beef easily decays during the storage.

Microorganism is the main cause of beef spoilage.[2] Different temperatures, packaging conditions, MAP (modified atmosphere packaging),[3] and preservatives significantly affect the microbial metabolism in beef.[4] Previous studies on beef spoilage were mainly focused on metabolisms. The quantity of microorganisms per unit area was commonly used to indicate the metamorphic grade.[5] Ellis *et al.* determined the spoilage of beef by detecting the change of the quantity of bacteria per unit area.[6] Amamcharla used FTIR spectrum to analyze the relationship between the viable counts of Salmonella enterica typhimurium and beef spoilage.[7] Xiao established the growth model of spoilage bacteria in fresh beef under MAP conditions.[8] This method based on the quantity of microorganisms requires the relatively long time and the detection cost is relatively high. It is not suitable for outdoor detection. For the microorganisms may change the appearance of the beef, some researchers used machine vision and viable image to measure the freshness of beef.[9–12]

Beef releases special volatiles during the spoilage, therefore we can discriminate its freshness with these gases. GC-MS (Gas Chromatograph-Mass Spectrometer) analysis was widely used to analyze the volatile compounds and detect alcohols, aldehydes, ketones, esters, and some sulfides. Argyri used the GC-MS method to analyze the volatiles under MAP conditions during beef spoilage. The analysis results revealed an increase trend of hexanol concentration and a mixed trend of aldehyde concentration.[13] Argyri used HPLC (High Performance Liquid Chromatograph) to analyze organic acids for determining the metamorphic status of beef.[14] The analysis results showed that the method could be used to determine the quality of beef, but it could not achieve the rapid detection. E-nose is another effective method for volatiles analysis. Blixt and Borch established the relationship between electronic nose signal and the spoilage status of vacuum-packaged beef.[15] Some researchers used the electronic nose for spoilage classification of beef.[16–19]

As a fast and non-destructive analysis method, FTIR (Fourier transform infrared) spectroscopy plays an important role in the detection of food spoilage. Zhao used FTIR spectrum to detect wine spoilage successfully.[20] For beef spoilage analysis, Ammor *et al.* used FTIR spectrum to detect the total viable count of bacteria and PH for determining the freshness of minced beef. The detection result showed that FTIR spectrum was a powerful method for the detection of minced beef.[21] Panagou collected and analyzed the FTIR spectrum to establish the correlation between partial least squares models and the total viable count of bacteria on beef surface.[22] Some more complex chemometrics algorithms were also used in to analyze the infrared spectra of beef and then to discriminate the spoilage.[23, 24] However, all of the studies above were based on infrared spectral analysis of beef surface but not volatiles.

FTIR is also an effective way to detect gaseous substances. It had been used to detect unknown gases in industry, foods and other fields. Xia used FTIR spectroscopy to detect aircraft engine emissions and FTIR spectroscopy showed the promising application prospects in the detection of aircraft engine emission.[25] Daham used FTIR spectroscopy to measure emissions of on-road vehicles.[26] We used conventional and long-path FTIR spectroscopy to measure volatiles of grape and strawberry and detected alcohols, esters, carbon dioxide, and other gases. The detection results showed that we could identify the spoilage of fruits according to the absorbance of these gases.[27, 28] Therefore, it is possible to analyze the volatiles from spoilage beef using infrared spectroscopy.

In this paper, we used long-path FTIR spectroscopy to detect and analyze the beef spoilage process. To our best knowledge, this is the first time to identify beef spoilage through the infrared spectra

of volatiles. The paper aims to measure special gases and analyze the variation of these gases during beef spoilage. We classified the collected spectra by using PCA (Principal Component Analysis). The paper provides a fast method for the detection and identification of beef spoilage.

2 Materials and methods

2.1 Experimental materials

Fresh beef was purchased from the Carrefour supermarket in the Haidian District, Beijing (China) and then brought to the laboratory within 30 min. We choose bovine tendon as the experimental material for it is common inmarket. Beef samples were cut into long belts (139 g) by a sterile knife and then 3 samples were directly put into the 1000-ml "Fuguang" (China) cup without washing. In order to study the change trend of the volatiles of different shapes of beef, we also put the other 3 samples of minced beef with the same weight to bovine tendon samples into the containers with the same capacity. All the samples were stored at room temperature (15 °C).

2.2 Experimental equipment

We used the Vertex 70 spectrometer (Bruker Company, Germany), MIR/FIR ceramic light source and MCT detector (liquid nitrogen cooling). The spectra were collected by OPUS Software 7.0 (Bruker Company, Germany) over the wavenumber range of 4,400~800 cm^{-1} and a resolution of 0.5 cm^{-1}. Cyclone™ C2 gas cell (Specac Company, England) was adopted in the experiment. We also used a 1-L vacuum pump (Feiyue, ALUE Company in Shenyang, China).

2.3 Experiment methods

Before the measurement, we connected the outlet and inlet of the gas cell respectively with the vacuum pump and the container (cup). In order to get a continuous and stable measurement, we added liquid nitrogen into FTIR spectrometer before the experiment. During the experiment, the volatiles in the cup were pumped into the gas cell. We used the OPUS Software 7.0 to collect the spectrum of beef samples at 9: a.m. and 9: p.m. every day. We also took the vacuum background photo during each collection. The 6 samples were stored at room temperature for 5 d. In the first two days of the experiment, the surface and smell of beef showed no significant change. On the third day, beef surface began to get dark and an irritating odor was generated. On the fourth day, beef deteriorated significantly.

2.4 Spectral data processing

We used the OPUS Software 7.0 to collect the original absorbance spectra. The baseline correction of spectra[29] was performed by OPUS 7.0. The smoothing of spectra[29] was completed by the SigmaPlot Software 12.0. We completed the analysis and forecast of PCA using the Unscrambler Software 9.7. The quantification analysis of the spectra were calculated by measuring the peak areas of the characteristic bands, after pre-processing.

3 Results and discussion

3.1 Spectral characteristic analysis of volatile gases from beef

The infrared spectra of fresh and decay beef (on the fifth day) are presented in Figure 1. Beef released large amounts of volatile during beef spoilage. The comparison results with the NIST spectra library indicated that the volatile might be ammonia.[30] The main absorbance band of ammonia is between 1200 cm^{-1} and 800 cm^{-1} and contains a lot of narrow absorption peaks. A lot of ammonia was detected during beef spoilage. However, little ammonia was detected in fresh beef, as shown in Figs. 1(a) and 1(b).

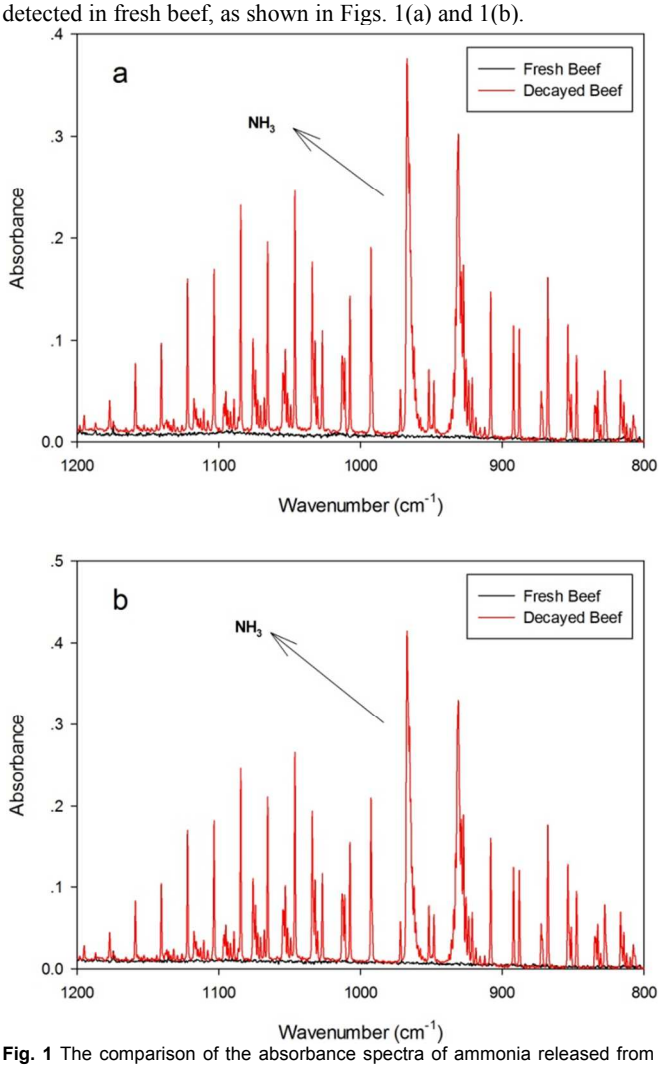


Fig. 1 The comparison of the absorbance spectra of ammonia released from fresh and decayed beef (—fresh beef, —decayed beef) a: bovine tendon, b: minced beef.

In addition to ammonia, beef also emitted alcohols, aldehydes, esters, ketones, and carbon dioxide in the process of spoilage, as shown in Figs. 2(a) and 2(b). Previous studies indicated that ketones were the components of volatile gases during the beef metamorphic process.[13] As shown in Fig. 3, the spectra between 1250 cm^{-1} and 1180 cm^{-1} may correspond to ketones in fresh beef and decay beef and corresponding absorbance is not changed significantly after beef spoilage.[30] We chose the wavenumber range of 2285-2180 cm^{-1} to analyze the change trend of carbon dioxide concentration. We did not choose the first peak of 2350 cm^{-1}, which was unsuitable for Lambert-Beer law because corresponding absorbance is beyond 0.3.[31] The change trends of carbon dioxide concentrations are shown in the Figs. 4(a) and 4(b).

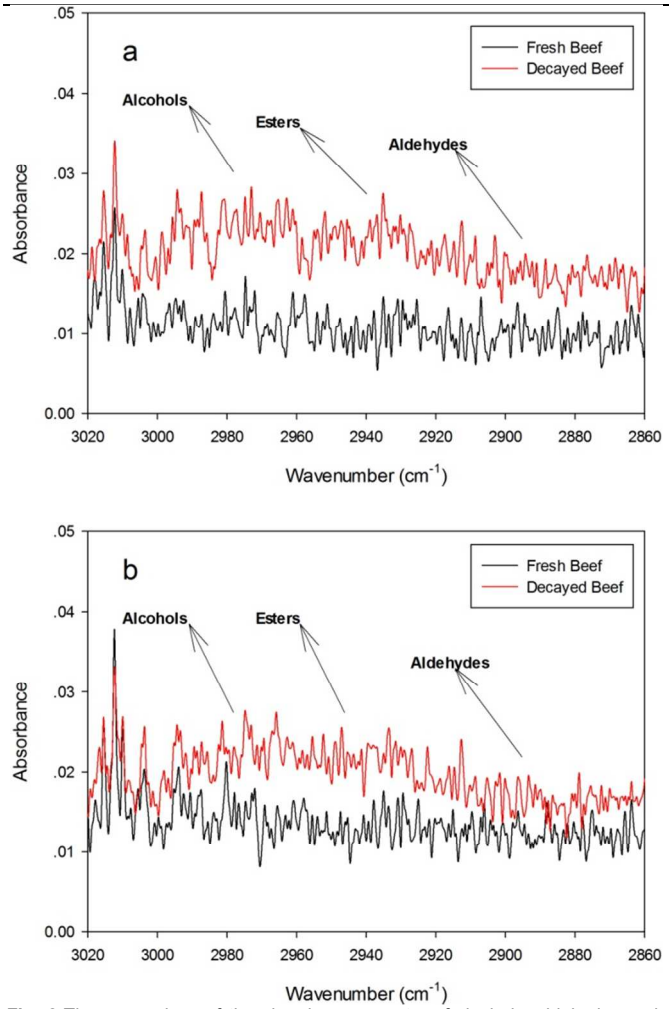
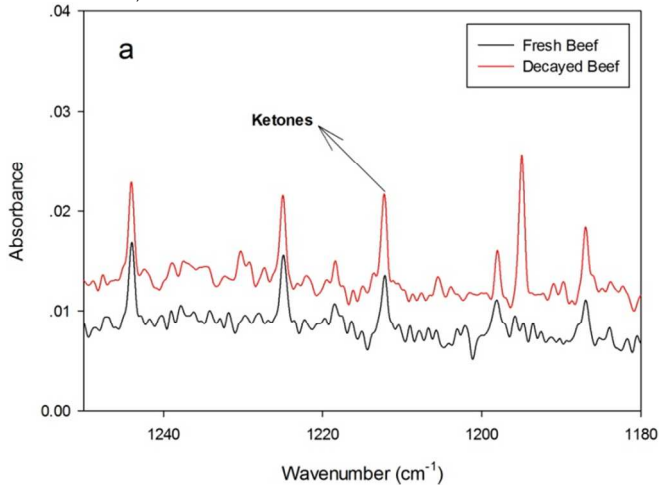


Fig. 2 The comparison of the absorbance spectra of alcohols, aldehydes and esters released from fresh and decayed beef(—fresh beef, —decayed beef) a:bovine tendon, b:minced beef.



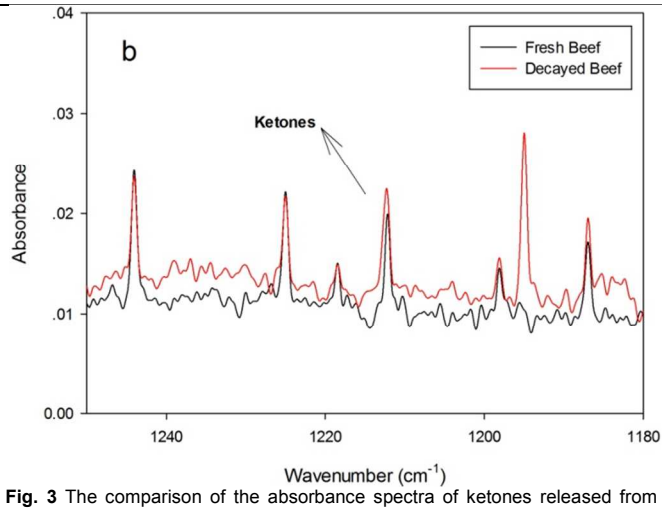


Fig. 3 The comparison of the absorbance spectra of ketones released from fresh and decayed beef (—fresh beef, —decayed beef) a: bovine tendon, b: minced beef.

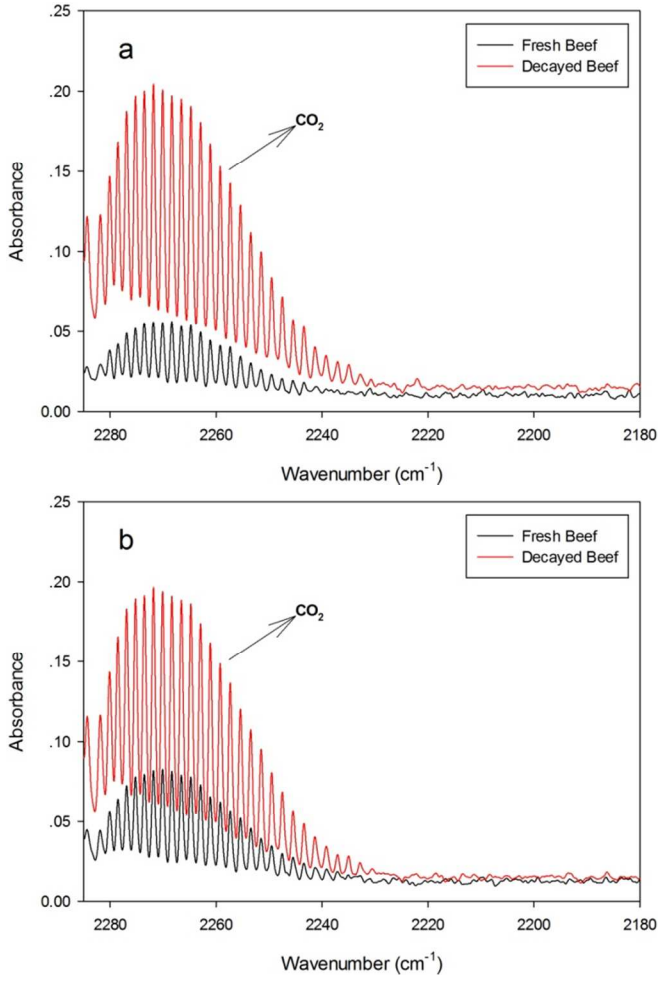
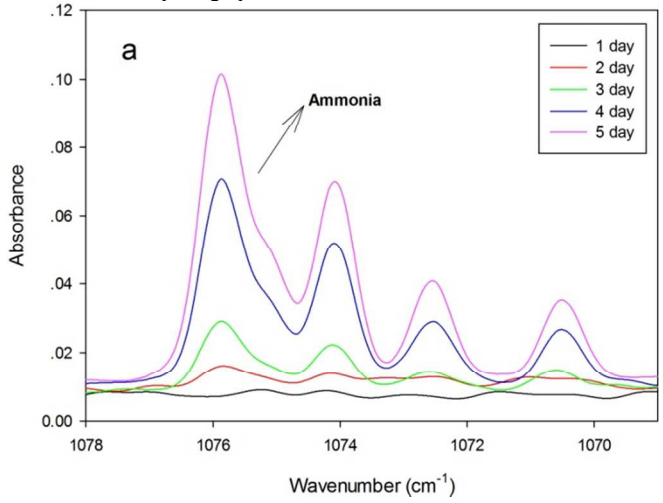


Fig. 4 The comparison of the absorbance spectra of carbon dioxide released from fresh and decayed beef (—fresh beef, —decayed beef) a: bovine tendon, b: minced beef.

3.2 Spectral analysis of the variation of the volatile gases from beef in different spoilage stages

According to the Lambert-Beer Law, the gas concentration is proportional to the absorbance under the constant optical path.[31] Therefore, we can quantitatively analyze the gas concentration according to the absorbance. We analyzed the spectral characteristics of ammonia, alcohols, and carbon dioxide and then determined the spoilage status of beef.

The peak area between 1078 cm^{-1} and 1069 cm^{-1} was chosen as the indicator of the ammonia concentration (Figs. 5(a) and (b)). We did not choose the peak at 966 cm^{-1} because the absorbance at 966 cm^{-1} was above 0.3 and not applicable to the Lambert Beer Law.[31] The peak at 966 cm^{-1} cannot reflect the change trend of the ammonia concentration correctly. We chose the peak area within the wavenumber range of 3020-2840 cm^{-1} as the indicator of alcohol concentration and obtained the change trends of the concentrations of ammonia and alcohols with the storage time. We used the peak area within the wavenumber range of 2285-2180 cm^{-1} to calculate the concentration of carbon dioxide. As shown in Fig. 1, the concentration of ammonia is gradually rising during beef spoilage. In the initial 24-h storage, the concentration of ammonia was not changed significantly, indicating that no obvious spoilage occurred on the surface of beef. Besides, the component types of volatile gases of bovine tendon were almost the same to those of minced beef. However, the concentrations of the above components showed distinct differences. In Figs. 6, 7 and 8, the concentrations of ammonia, alcohols, and carbon dioxide from minced beef were higher than those from bovine tendon because the contact area between minced beef and air was larger than that between bovine tendon and air. The microbial activities in minced beef are more significant than that in bovine tendon. These above factors accelerated the spoilage process of beef.



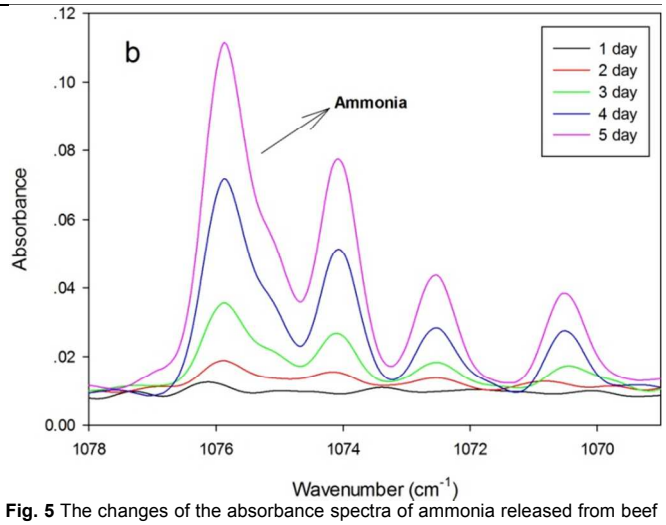


Fig. 5 The changes of the absorbance spectra of ammonia released from beef during spoilage (—Day 1, —Day 2, —Day 3, —Day 4, —Day 5) a: bovine tendon, b: minced beef.

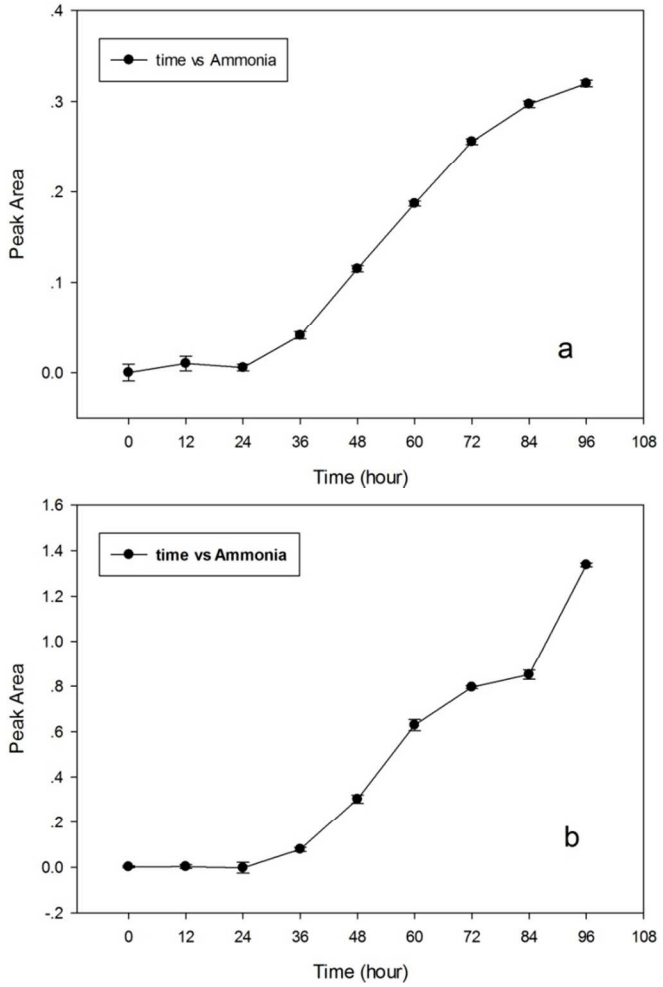


Fig. 6 The changes of the concentration of ammonia released from beef during spoilage in 5 days (a:bovine tendon, b:minced beef).

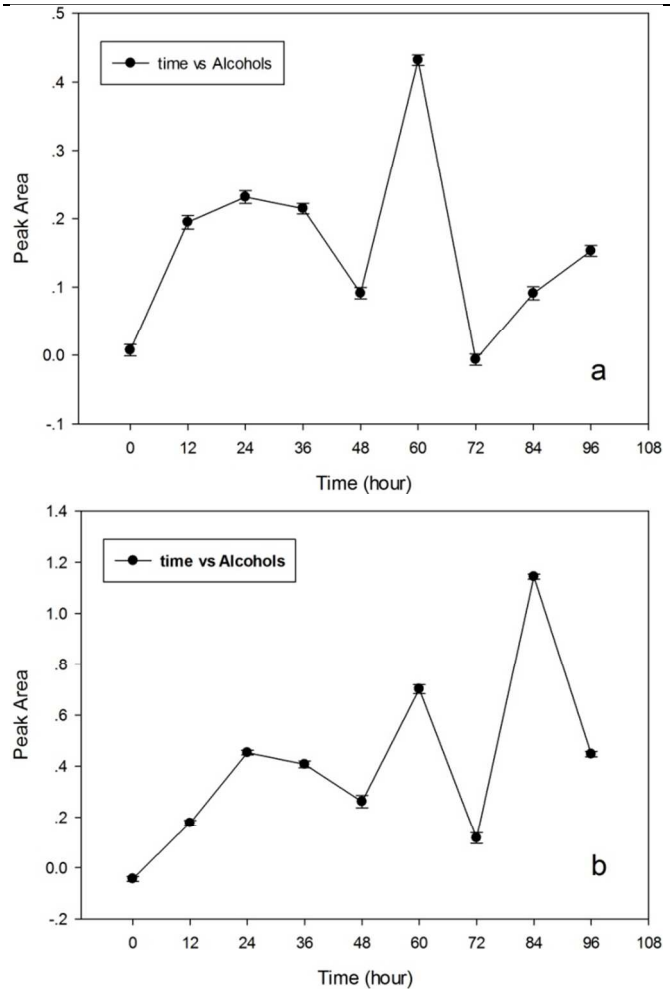
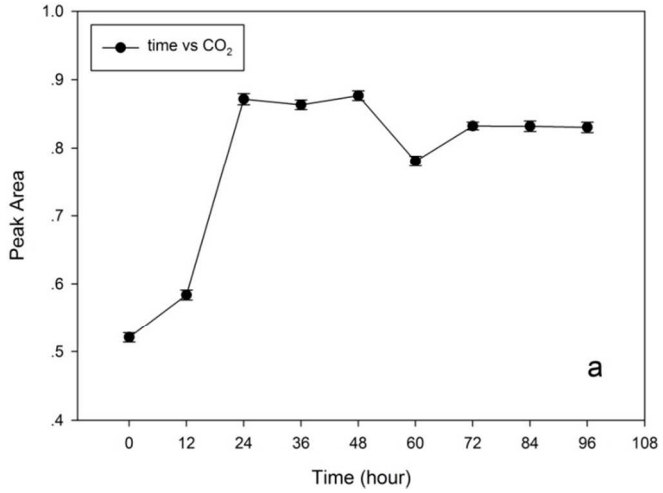


Fig. 7 The changes of the concentration of alcohols released from beef during spoilage in 5 days (a: bovine tendon, b: minced beef).



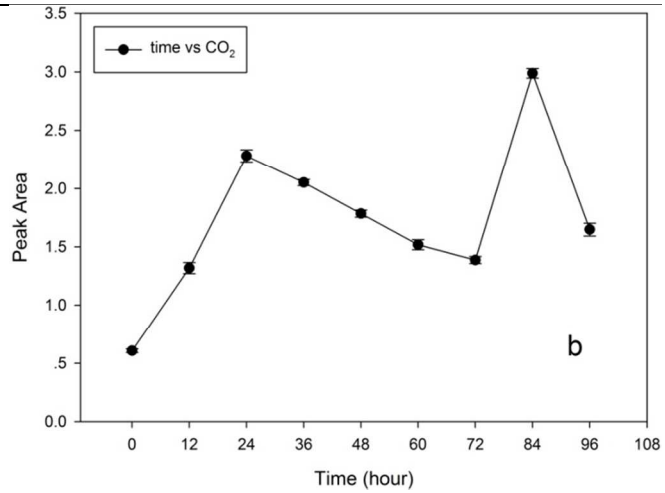


Fig. 8 The changes of the concentration of carbon dioxide released from beef during spoilage in 5 days (a: bovine tendon, b: minced beef)

The concentrations of ammonia, alcohols, and carbon dioxide were calculated (Figs. 6, 7, and 8). The concentrations of volatile gases vary with the storage time. The increase trend of the ammonia concentration was more significant than that of the concentration of alcohols. Especially in the storage period from the 24th h to the 72nd h, the ammonia concentration showed a significant increase and beef spoilage was accelerated. Carbon dioxide concentration exhibited a similar increase trend consisting of the initial 24-h rapid increase, the subsequent relatively small change, and the stable variation without significant increase. The main reason is that in the initial stage the dominant microbes mainly belong to aerobic respiration microbes and generate a lot of carbon dioxide. In the final storage stage, with the decrease in the oxygen concentration, the dominant microbes become anaerobic respiration microbes and produce a small amount of carbon dioxide. The concentration of carbon dioxide was relatively stable in the final storage stage. The changes of carbon dioxide concentration are shown in Figure 8. The error bar was calculated with six consecutive measurement values. Figure 7 shows the concentration change trends of alcohols. In the initial 24 h, the concentrations of alcohols increased dramatically. The change may be interpreted as follows. Firstly, a lot of aerobic bacteria and anaerobic bacteria occurred during beef spoilage and the anaerobic bacteria produced alcohols, which were then oxidized and transformed into other substances. The above results indicate that the infrared spectra can be used to distinguish fresh beef from spoiled beef.

3.3 PCA analysis of the spectra of beef volatiles during spoilage

The concentrations of various volatile gases generated during beef spoilage showed different change trends. We analyzed the data using PCA for dimension reduction and then explored the discrimination capability of infrared spectroscopy for spoiled beef. In the 5 days of the experiment, beef was fresh in the first two days. We classified 1- and 2-day old beef as fresh beef, 3- and 4-day old beef as the slightly decayed beef, and the 5-day old beef as the seriously decayed beef. We treated the period from the morning of the 3rd day to the evening of the 4th day as the spoilage transition period. In order to get clear classification results, we removed the data of the evening of the 2nd day, the morning of the 3rd day and the evening in the 4th day. The data from the wavenumber range of 1078-1069 cm^{-1} were selected as the variable for PCA analysis. The number of principal components is selected to be 20. According to the analysis results (Fig. 9), the three samples can be

easily distinguished from each other. For bovine tendon (Fig. 9a), PC1 explains 95% variance and PC2 is 5%, while for minced beef, PC1 and PC2 represent 94% and 5%, respectively. Some individual samples show certain discrete results compared with the overall clustering region, which may be caused by the operation errors in the sampling process. It is also can be studied from Fig.9 that PC2 scores are similar for the three group of samples while PC1 scores are obviously different. Actually, PC1 represents the spectral characteristics that increase with storage time.

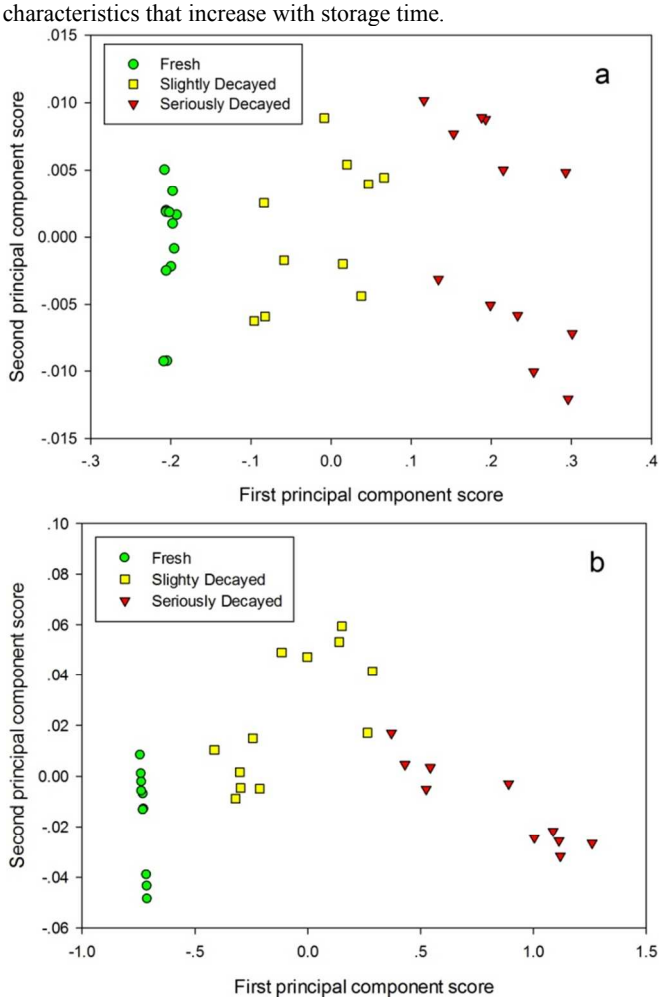


Fig.9 The PCA scores of fresh and spoilage beef samples (a: bovine tendon, b: minced beef).

The infrared spectra of the volatiles of fresh beef, slightly spoiled beef, and seriously spoiled beef showed significant differences. According to the classification results based on the concentration change trend of ammonia released from bovine tendon, fresh beef, slightly spoiled beef, and seriously spoiled beef can be clearly distinguished from each other. The results were also applied to minced beef.

4. Conclusions

Previous traditional discrimination methods of beef spoilage were mainly based on the analysis of the total viable counts of bacteria . In this study, we used long optical-path infrared spectroscopy to measure the characteristic volatiles released from beef and then to discriminate the spoilage. During the beef spoilage process, we detected ammonia, alcohols, carbon dioxide, and other volatile gases. Based on the infrared spectra and the chemometric methods, the spoilage of fresh beef, slightly spoiled beef, and seriously spoiled beef can be easily discriminated. Compared with previous study, the method proposed in this study is a gas-phase based infrared spectral analytical method with advantages of no pre-process, fast, non-contact and high sensitive. It should be noted that the experimental system in this study is complex and expensive, but it can be simplified to a multi-bands laser spectroscopy system when the spectral characteristics are known. For example, we can use a DFB laser with ammonia characteristics band as a light source to establish a tunable diode laser spectroscopy system to measure the ammonia concentrations and then to discriminate the spoilage status of beef, and this will lower the system cost.

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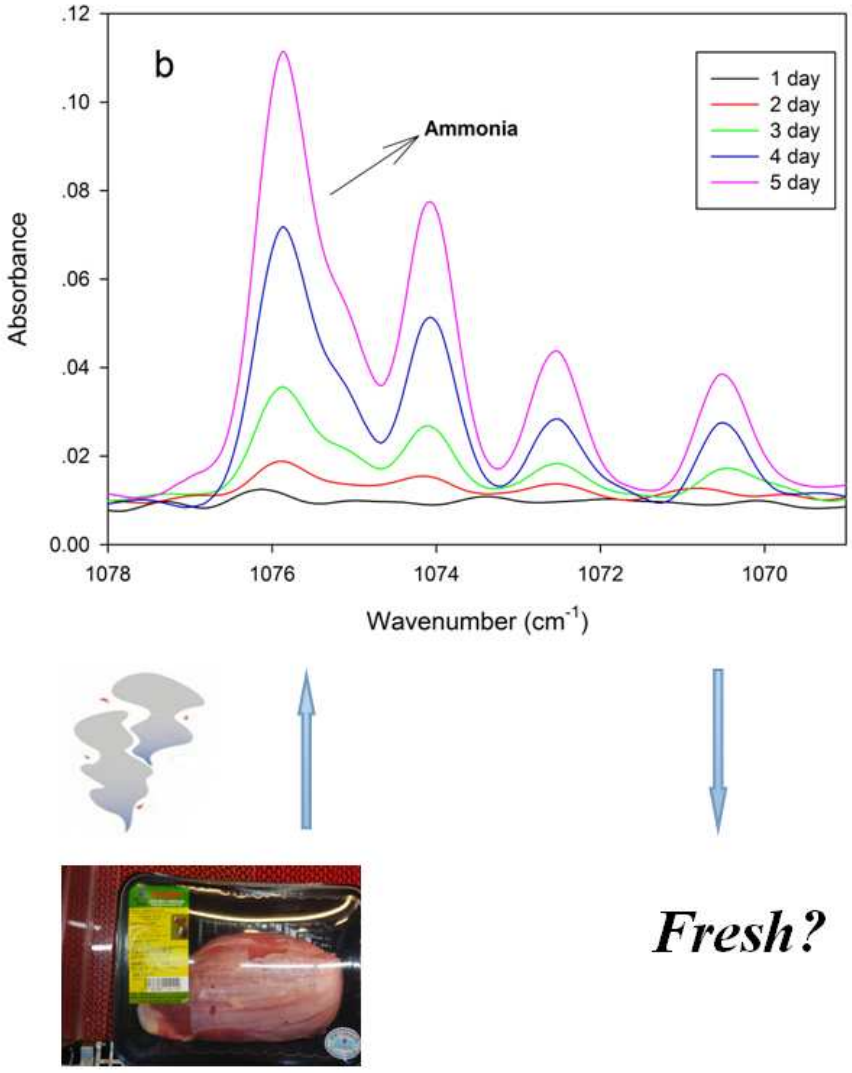

The freshness of beef was analyzed and indentified *via* its volatiles using long optical-path infrared spectroscopy. The spectral characteristics of some compounds, especially ammonia, were observed and demonstrated to have obvious differences between fresh and decayed beef.