Efficient Detection of Edible Oils Adulterated with Used Frying Oils through PE-film-based FTIR Spectroscopy Combined with DA and PLS

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Abstract: Edible oil adulteration has been a considerable problem worldwide, and rapid detection methods should be established. In this study, a validation method for edible oil adulterated with used frying oil (UFO) was introduced through Fourier transform infrared (FTIR) spectroscopy. The spectral region of 6000–400 cm\(^{-1}\) was determined through FTIR by using a disposable polyethylene film, and absorption profiles at 1550–650 cm\(^{-1}\) region could be used for detection analysis. A qualitative analysis model was established through discriminant analysis, and edible oil adulteration with more than 1% content of UFO could be qualitatively analyzed. A quantitative analysis model was also created through partial least squares regression. When the actual value was more than 1.5%, the predicted and actual values showed good linear correlation. FTIR coupled with chemometric analysis is a useful tool to detect edible oil adulteration.

Key words: edible oils, FTIR, used frying oil, adulteration

1 Introduction

Frying is a common food processing method by which food can be properly and quickly cooked. Nevertheless, harmful chemical reactions, such as oxidation, polymerization, thermal decomposition, and hydrolysis, occur during frying; consequently, many byproducts, such as free fatty acids, alcohols, polymers, and acrylamides are produced\(^{1-2}\). With high costs and increased oil price, fraud exists in oil industry worldwide. For example, many adulterations are caused by the addition of edible vegetable oils with low nutritional and commercial values into high prices oils. Unscrupulous businessmen also adulterate high-quality oil with used frying oil (UFO), which is a potential health risk for consumers.

Methods have been reported to assess adulteration in qualified vegetable oils based on the physical properties or chemical components because adulteration is difficult to evaluate with naked eyes. Instrumental analytical methods are also used to analyze the chemical composition of samples according to chemical or physical properties. Detection of adulteration in edible oils is commonly conducted through chromatography\(^{3}\). However, chromatographic separation is time consuming, and many expensive standards are required\(^{4,5}\). Analytical methods for the detection of adulterants in fats and oils mainly include differential scanning calorimetry (DSC), Raman spectroscopy, synchronous fluorescence spectroscopy, electron spin resonance, and nuclear magnetic resonance\(^{6-10}\). All of these methods exhibit specific drawbacks, such as intensive labor, complex operation, time-consuming sample preparation, and highly toxic chemical reagents. For instance, DSC requires a relatively expensive analytical instrument, and it can analyze samples in terms of thermal behaviors. Some studies have shown the ability of DSC coupled with principal component analysis (PCA) to discriminate edible oil adulteration even at a low mixed dose of less than 0.5%, but they have failed to quantify adulterants at a dose of less than 0.5%\(^{6,17}\). Satisfactory effectiveness in adulteration detection can be obtained by combining these methods with chemometric analytical techniques, such as PCA, linear discriminate analysis, partial least squares (PLS), and multiple linear regression\(^{17-22}\). Thus, rapid and simple detection methods should be established.

Fourier transform infrared (FTIR)/spectroscopy is a promising technology for the food industry because of its simple, rapid, and nondestructive measurements\(^{23,24}\). It can reduce the use of labor and reagents, and its application in edible oil analysis has increased. Several studies have also extensively evaluated FTIR application in terms of its ability to determine fatty acid composition in marine...
oils and vegetable oils as well as phospholipid contents in vegetable oils\textsuperscript{35–37}. FTIR is also utilized to determine acid and peroxide values of edible oils based on the O–H stretching band and to analyze the thermal stability of edible oils\textsuperscript{28–30}. The application of FTIR in the analysis and characterization of adulterated oils and fats has also been explored\textsuperscript{31, 32}. Elzey \textit{et al.} combined FTIR with PLS multivariate regression analysis to determine the compositions of natural oils adulterated with neem oil and flaxseed oil and obtained a detection limit of 10\%\textsuperscript{31}. Rohman \textit{et al.} showed that pure extra virgin olive oil (EVOO) and a mixture of olive oil with palm oil (PO) as an adulterant are precisely classified into two groups, but this model is used for adulterated EVOO with PO only\textsuperscript{32}. Zhang \textit{et al.} discriminated oil adulteration, and adulteration can be distinguished when the proportion is 2\%\textsuperscript{18}. However, sample preparation is considerably complicated for pellet pressing, which is labor intensive and time consuming. Besides, this study was only used one UFO and four vegetable oils as test materials, the oil sample’s representative is lacking. For single edible oil, it is easy to distinguish the adulteration with UFO due to the differences in chemical composition and properties between them\textsuperscript{31}. Our research group developed a new technique for spectral acquisition by using a polyethylene (PE) film, and the application of PE film-based measurements can simplify and facilitate low-cost FTIR analyses\textsuperscript{4, 30}.

Considering these results, we mainly aimed to distinguish between edible oil and oil adulterated with UFO by establishing a UFO identification model and a quantitative detection model with PE-based FTIR spectral data of edible oils, UFOs, and adulterated oils. This study was also designed to determine the accuracy of the proposed model through an actual test. We hope it can provide a basis for the rapid and accurate determination of the adulteration of edible oils.

2 Materials and Methods

2.1 Materials and reagents

Thirty-one edible oils, including blended oil and oil from rapeseed, soybean, peanut, sesame, virgin olive, pepper, sunflower, wild camellia, perilla seed, bitter apricot kernel, and mustard, were purchased from local supermarkets in Yangling, Shaanxi, China. Eleven UFO samples were collected from seven different cities in China. A transparent PE film, which was approximately 0.025 mm in thickness, was obtained from a local market.

Either absolute ethyl alcohol, chloroform, potassium hydroxide, sodium silicate, potassium biphthalate, phenolphthalein, hydrochloric acid, potassium iodide, sodium thiosulfate, salicylic acid, potassium dichromate, and activated clay were purchased from Tianjin Chemical Company, Ltd. All reagents and chemicals were of analytical grade.

2.2 Sample preparation

UFO samples were filtered through vacuum suction to remove impurities. The peroxide and acid values of the samples were measured (Table 1), and the obtained values were consistent with the standards\textsuperscript{33}.

On the basis of the mass ratio of 1:1 (two oils), we randomly selected the samples from 31 kinds of edible oil samples to produce eight different blends of edible oil samples. In this manner, 39 edible oil samples were prepared. Similarly, 11 UFOs were allocated randomly to produce four blends of UFO samples, resulting in 15 UFO oil samples. Randomly selected edible oils were adulterated with different UFOs at varying levels (0.1\%–15\%, w/w), which were marked as 0.1\%, 0.2\%, 0.3\%, 0.4\%, 0.5\%, 0.6\%, 0.7\%, 0.8\%, 0.9\%, 1\%, 1.1\%, 1.3\%, 1.5\%, 2\%, 3\%, 4\%, 5\%, 6\%, 7\%, 8\%, 9\%, 10\%, 11\%, 12\%, 13\%, 14\%, and 15\%. A total of 192 samples were obtained: 39 samples were pure edible oil, and the remaining ones were adulterated oil samples.

2.3 Spectral acquisition and processing

A Bruker VERTEX 70 series FTIR spectrometer (Bruker Optics, Germany) equipped with a deuterated triglycine sulfate detector was used. At ambient temperature (23\textdegree C \pm 2\textdegree C), 10 g of oil sample was dissolved in 10 g of isooctane. Approximately 100 \textmu L of sample was deposited onto the surface of the transparent PE film (1 cm\textsuperscript{2}) and subsequently spread uniformly by using a micropipette\textsuperscript{4}. The solvent was evaporated to form an oil film, and films prepared in this manner were maintained in a horizontal position. The spectra of the oil film were obtained using a PE film background spectrum by utilizing an FTIR spectrometer with wavelengths ranging from 6000 cm\textsuperscript{-1} to 400 cm\textsuperscript{-1}. The FTIR spectrometer was operated in a transmission mode, and each recorded spectrum was obtained by determining the average of 16 scans at a resolution of 4 cm\textsuperscript{-1}. An effective pathlength was identified from each spectrum and used to normalize the spectrum to a fixed pathlength (0.15 mm). This effective pathlength could be used to compare results from different films or various sample loadings quantitatively.

| Table 1 Acid and peroxide values of oil samples. |
|-----------------|---------------|---------------|
| Oil samples     | Acid value/   | Peroxide value/ |
|                 | mgKOH/g       | mmol/kg       |
| Edible oil      | 0.02–1.70     | 0.43–7.84     |
| UFO             | 0.08–2.80     | 0.40–9.14     |
| Adulterated oil | 0.20–1.60     | 0.95–3.30     |
2.4 Model establishment

Forty randomly selected oil samples were pretreated and scanned to obtain the spectra. Spectral data processing was carried out, and a UFO identification model was established using TQ Analyst 7.2 (Thermo Electron Inc., Madison, WI).

A total of 151 samples, including 27 randomly selected edible oils and 124 randomly selected adulterated oils were pretreated and scanned to obtain the spectra for a UFO quantitative detection model, which was established using TQ Analyst 7.2.

2.5 Validation

A total of 23 randomly selected oil samples, which were not involved in UFO identification model establishment, were used as validation samples to verify the reliability of the UFO identification model.

In addition to the 151 samples used to establish the model, the remaining 41 samples were used to determine the reliability of the UFO quantitative detection model.

2.6 Statistical analyses

A qualitative analysis model was established through discriminant analysis, and its evaluation standard was the sample recognition rate. A quantitative analysis model was created by PLS method, and the evaluation standard included the correlation coefficients and standard deviations (SD) between the predicted and measured values.

3 Results and Discussion

3.1 Spectral analysis

The spectra obtained with PE-based FTIR are presented in Fig. 1. In Fig. 1, several distinguishing characteristics, such as the strongest peak at 1127–1072 cm\(^{-1}\) corresponding to the stretching vibration of C–O groups in ester bond, were observed. According to Guillen and Cabo\(^{36}\), the frequencies of some bands in the fingerprint region depend largely on sample composition. Knowledge on the relation between the FTIR spectrum of oil and its chemical component structures helps elucidate the spectra. A series of complex chemical reactions, such as oxidation, hydrolysis, isomerization, and polymerization, occurred during food frying, especially repeated and prolonged deep frying. Triacylglyceride (TAG) degradation products, including free fatty acids, mono- and diacylglycerols, and glycerols, mainly resulted from breakages in the carbon–carbon double bond and ester bond. With the reduced ester bonds attributed to TAG degradation, the signal intensity of UFO detected at 1127–1072 cm\(^{-1}\) was remarkably lower than that of edible oils, and the signal intensity of adulterated oil was found between their intensities. The peak at 1390–1371 cm\(^{-1}\) was primarily ascribed to the stretching vibration of C–H bonds (\(\text{CH}_3\)) in the oils. Other peaks at 1480–1390 cm\(^{-1}\) were also ascribed to the stretching vibration of C–H bonds in other groups, such as the C–H bonds of olefinic carbon. Thus, the height of peaks at 1390–1371 cm\(^{-1}\) of edible oil was higher than that of UFO because of hydrogen substitution in complex chemical reactions during frying. The characteristic absorption peaks at 1550–650 cm\(^{-1}\) were evident, and they could be used for detection analysis. No significant differences were observed among these three spectra in the absorption peak positions and profiles. Nevertheless, subtle discrepancies existed among these spectra. Small differences were also observed in the absorption intensities of the same peak. Therefore, adulterated oils could be distinguished in terms of the absorption band position and absorption intensity of the same band.

![Fig. 1](image-url)

Fourier transform infrared spectra of oils. (a) Spectral region of 4000–400 cm\(^{-1}\). (b) Spectral region of 1050–650 cm\(^{-1}\).
3.2 Qualitative analysis of adulteration

When UFO was added to fresh edible oils, the chemical composition of the oils changed. Different chemical substances contain various functional groups. Changes in compositions appeared in the FTIR spectral discrepancy between fresh edible oils and adulterated oils. On the basis of the differences between edible oils and adulterated oils, we used 40 samples as a calibration set to establish the qualitative analysis model through discriminant analysis by utilizing the FTIR spectra at 1550–650 cm$^{-1}$. The discriminant analysis result (Fig. 2) was acceptable, and the UFO and edible oil were strictly divided into two categories. The UFO was easily distinguished from the edible oil by utilizing the qualitative analysis model with a recognition rate of approximately 100%. Adulteration could be distinguished when the proportion was as low as 1%, which was a satisfactory discriminant analysis result. Using this method, we could efficiently qualitatively analyze adulteration.

To examine the reliability of the model, we analyzed 23 randomly selected samples, which were not involved in model establishment, by applying the same procedure. In Table 2, 100% recognition rate indicated that the UFO identification qualitative analysis model was feasible.

The model was established by the spectral region at 1550–650 cm$^{-1}$, not just by the amounts of polar components and polymers or other indicators. Although this interval mainly reflected the amounts of functional groups such as carbonyl and ester bonds, the establishment of the model involved all the properties of samples shown at 1550–650 cm$^{-1}$. The different UFOs used to establish model did have compositional differences, however, multiple UFOs were used together to build the model, so that the differences in the spectrum could be captured by software and the model could accurately distinguish between the edible oils and UFOs.

3.3 Quantitative analysis of adulteration

3.3.1 Model establishment

On the basis of the spectral region at 1550–650 cm$^{-1}$ obtained with PE-FTIR, we created the quantitative analysis model through PLS method. The predicted adulteration proportion of the calibration set was calculated using the equation of quantitative analysis. The actual and predicted values were used to construct linear regression equations with the calibration set. The simple distribution of the scatter plots between the actual and predicted values of concentration was obtained (Fig. 3). Significant linear correlations were observed between these two variables, with a correlation coefficient of 0.9822, a slope of 0.9819, and an intercept of 0.0015. These values indicated that the predicted value was close to the actual one. SD was 0.2389, which showed that the accuracy was ± 0.24% within the concentration of 0%–15%. These results suggested that the model was available, and it could be used to further determine the blending concentration.

3.3.2 Model validation

The model was validated with 41 samples that were not involved in the model establishment to verify its reliability. In Fig. 4, when the actual concentration was less than 1.5%, the points were scattered, and the linear regression was difficult to obtain. This result could be explained by assuming that the small change in the chemical components of the different adulteration proportions caused a

<table>
<thead>
<tr>
<th>Oil samples</th>
<th>Number of oil samples</th>
<th>Recognition rate/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verification</td>
<td>23</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2 Results of the identification calibration of UFO and adulterated oil.

Fig. 2 Qualitative analysis model established by discriminant analysis.

Fig. 3 Linear relationship between predicted and actual concentrations in a calibration set.
large error between the predicted and actual values. In Fig. 4(b), when the actual concentration was more than 1.5%, the linear correlation between the predicted and actual values was good, with $R^2$ of 0.9883 and a slope of 0.9165, which was close to 1. The predicted value was almost equal to the actual value, and SD was 0.1655, indicating that the model was feasible. Therefore, the quantitative analysis model based on the PE-FTIR spectral acquisition could quantitatively detect adulterated concentration. The blending concentration was more than 1.5%. Similar to qualitative analysis, quantitative analysis could be achieved by the spectral region at 1550–650 cm$^{-1}$. We could not explain for the time being how it specifically quantify adulteration. But there are certainly modules within the model that can identify and quantify adulteration, thus a good result of model validation was obtained.

### 3.3.3 Error analysis

Actual and predicted values were compared with those obtained through paired t-test to confirm the precision and accuracy of this method. Our results revealed a t-value of 1.06, which was less than the critical value ($t_{19,0.05} = 1.972$), and a P-value of 0.290, which was higher than 0.05, indicating that the actual and predicted values did not significantly differ, and the proposed method was feasible.

One of the samples was optionally scanned 10 times to validate the reproducibility of regression analysis. The relative standard deviation of the predicted values was 1.58%. Ten randomly selected samples with a concentration of more than 1.5% were determined, and the relative error ranged from 0.32% to 7.64%, which was less than 10%. These data verified the accuracy and reproducibility of this model.

Elzey et al., Rohman et al. and Zhang et al. investigated the adulteration of oil, and all of their established methods display specific defects$^{18,31,32}$. A detection limit of 10% was slightly high, and spectral acquisition is used by KBr pellet, sample preparation was considerably complicated, which was labor intensive and time consuming. Signals were more than 2 times greater than the literature by using PE-FTIR technique. The stronger signals could directly increase detection accuracy. Besides, the test materials in these studies were relatively less and lacked representative. However, the proposed method involved a disposable PE film as a spectral acquisition accessory, which considerably simplified the test process. In addition, most detection methods in other studies were applicable to adulteration between specific oils. In the present study, a wide range of options for edible oils included most common types, but the type of adulterated oil cannot be determined without prior detection. Therefore, the proposed method was remarkably practical in the detection of adulterated oil.

### 4 Conclusion

This study developed an efficient detection method by increasing the amount of oil samples to improve the stability of the detection model. The proposed method rapidly detected UFO and provided advantages of convenient operation, high detection accuracy, and non-pollution, thereby replacing complex and costly traditional methods. According to the PE-FTIR spectra at 1550–650 cm$^{-1}$ and the results of discriminant analysis, edible oil adulteration with more than 1% content of UFO could be qualitatively analyzed, and the recognition rate could reach 100%. Samples with concentrations of more than 1.5% were applied to the quantitative analysis model established through PLS, our results revealed good reproducibility and accuracy.
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