Authentication of *Eucommia ulmoides* Seed Oil Using Fourier Transform Infrared and Synchronous Fluorescence Spectroscopy Combined with Chemometrics

Keqing Hu¹, Zongyao Huyan¹, Syed Tufail Hussain Sherazi², and Xiuzhu Yu¹*  

¹ College of Food Science and Engineering, Northwest A&F University, 22 Xinong Road, Yangling 712100, Shaanxi, P. R. CHINA  
² National Center of Excellence in Analytical Chemistry, University of Sindh, Jamshoro, 76080, Sindh, PAKISTAN  

**Abstract:** *Eucommia ulmoides* is a traditional Chinese herb whose seeds can be used to produce edible oils. Fourier transform infrared (FTIR) and synchronous fluorescence spectroscopic (SyFS) spectra of *Eucommia ulmoides* seed oil (EUSO) are lacking. The relevant functional and fluorescent groups were determined by FTIR and SyFS techniques for discriminating adulteration of EUSO, respectively. FTIR and SyFS spectra of EUSO and six common-used vegetable oils were recorded from 4000–400 cm⁻¹ and 250–700 nm at wavelength interval of 60 nm, respectively. Principal component analysis (PCA), linear discriminant analysis (LDA), cluster analysis (CA) and partial least square (PLS) regression was used for qualitative and quantitative calibration of EUSO adulteration. The FTIR spectral regions of 1429–1377 cm⁻¹ and 1128–1110 cm⁻¹ based on PCA, LDA, and CA, and the PCA of SyFS spectral regions of 600–700 nm and 300–500 nm were evaluated for qualitative differentiation of EUSO adulteration. The recognition rate of PCA validation was found to be 100% by FTIR regions. PLS calibration was optimal by the spectral normalization vector treatment in the two FTIR spectral regions and SyFS spectra were combined with characteristic absorption peak area, which can achieve quantitative detection of EUSO adulteration. The two techniques are useful for EUSO adulteration detection at levels down to 1% and 0.48% (w/w), respectively. The results indicated that spectral information obtained by FTIR and SyFS of EUSO can be used for qualitative and quantitative analysis of EUSO adulteration with the advantages of high sensitivity, simplicity, and rapidness.

**Key words:** *Eucommia ulmoides* seed oil, FTIR, fluorescence spectroscopy, authentication

1 **Introduction**

*Eucommia ulmoides* Oliver (*E. ulmoides*) belongs to a single-species genus of the family Eucommiaceae. In Chinese, *E. ulmoides* Oliver is known as Du-Zhong. It is one of the most commonly used traditional Chinese herbs in Asian countries⁴. A number of studies reported that many bioactive components are present in *E. ulmoides* serve as antioxidant, antimicrobial, and anti-inflammatory agents, such as polyiridoid glycosides, aucubin and chlorogenic acid⁵. Therefore, its food products have been recognized as functional foods⁶. *E. ulmoides* seed oil (EUSO) is an important product and can serve as cooking oil or dietary supplement. It contains high amounts of polyunsaturated fatty acids and bioactive components, such as α-linolenic acid that has many health benefits, including cardioprotective effects, positive influence on central nervous system functions, and modulation of inflammatory responses. EUSO contains α-linolenic acid and total tocopherols more than 60% and 1000 mg/kg, respectively⁷.⁸. The study on spectra of EUSO is scanty. Hence, it is essential to collect the spectra of EUSO which can be used for developing rapid methods of EUSO determination.

Currently, chromatographic and spectroscopic methods, such as gas chromatography (GC) and high-performance liquid chromatography, are widely used to evaluate edible oils⁹.¹⁰. The levels of some triacylglycerols, sterols, and fatty acids can be utilized as biomarkers to detect oil adulteration and characterize the different blends of edible oils¹¹. However, these methods involve complicated pretreatment of oil samples and require a large amount of solvent and expensive instruments. Therefore, these techniques are unsuitable for rapid differentiation. Spectroscopic methods, such as infrared (IR) and fluorescence, are preferred as alternative means due to their time-saving and...
non-destructive nature\textsuperscript{12--14}. Fourier transform IR (FTIR) spectroscopy determines functional groups by absorption of IR radiation due to characteristic absorption bands at specific regions\textsuperscript{35}. Numerous works have reported its application in authentication of fats and oils\textsuperscript{[7]}. Vegetable oils contain several fluorescent groups, such as tocopherols, sterols, and chlorophyll. Therefore, fluorescence spectroscopy is also widely employed for characterization and differentiation of oils and fats\textsuperscript{16}. Both FTIR and fluorescence spectroscopic techniques can be used to achieve desirable results\textsuperscript{17,18}. In the present study, FTIR and SyFS spectra of EUSO and other vegetable oils were analyzed based on the functional groups and fluorescence chemicals. Combination and comparation of FTIR and SyFS associated with partial least square (PLS) regression, principle component analysis (PCA), cluster analysis (CA) and linear discriminatved analysis (LDA), as well as qualitative and quantitative analysis were developed to authenticate and predict EUSO adulteration rapidly.

2 Materials and Methods

2.1 Materials

EUSO, refined rapeseed oil (RRO), crude rapeseed oil (CRO), peanut oil (PO), corn oil (CO), cotton seed oil (CSO), and sunflower seed oil (SSO) were obtained from a local supermarket in Yangling, China. All botanical origin and oil quality were guaranteed by manufacturers. Transparent food wrap (0.025 mm thick polyethylene (PE) films (food grade)) was purchased from local supermarket. Chromatography-grade standards of hendecanoic acid glycerides were obtained from Tianjin Chemical Company Ltd. (Tianjin, China).

Adulterated EUSO samples were prepared by direct mixing of EUSO with six different low-priced vegetable oils at different amounts: 0%, 0.5%, 1%, 2.5%, 5%, 10%, 20%, 30%, 40%, 50%, and 100% (w/w). EUSO and 60 adulterated samples were used as calibration set and 26 adulterated samples with different ratios (which were prepared randomly at different adulterated amounts: 0%, 0.5%, 1%, 2.5%, 5%, 10%, 20%, 30%, 40% and 50% (w/w)) were used as validation set. All experiments and calculations were carried out in triplicate.

2.2 Spectra acquisition

FTIR spectra were acquired using a Bruker VERTEX 70 series FTIR spectrometer equipped with a deuterated triglycine sulfate detector. All spectra were recorded at ambient temperature and approximately 100 μL of oil samples was deposited onto the surface of a PE film, which was mounted on the transmission cell holder. All spectra were collected by the co-addition of 32 scans at a resolution of 4 cm\textsuperscript{-1} with PE film as the background. The effective path lengths of oil film spectra were normalized to a fixed path length of 0.15 mm by using a path length calibration equation that relates the effective path length to the absorbance at 4334 cm\textsuperscript{-1}\textsuperscript{46}. The spectral characteristic peaks related to authentication were analyzed after path length normalization.

All fluorescence measurements were performed using a Perkin–Elmer model LS–55 luminescence spectrometer, which is a fully computer-controlled instrument equipped with a 20 kW xenon discharge lamp as the light source and excitation and emission monochromators. The difference between the excitation and emission wavelengths Δλ was set at 60 nm, and the widths of excitation and emission were 10 nm. The intensities of SyFS spectra were recorded from 250–700 nm. The samples were shaken to mix before analysis. Then, it was directly dispersed into a 10 × 10 × 45 mm quartz cell for data collection and reading using FL Winlab v4.00.03. Every sample was scanned twice. To avoid spectroscopic distortions, spectra were corrected for the excitation lamp, the photomultiplier detector spectroscopic response, and emission and excitation gratings\textsuperscript{19}. Spectral plots were plotted using Origin Pro 9.0 (Origin Lab Corporation, Northampton, MA, USA).

2.3 Fatty acid profile analysis

Fatty acid compositions of oils were determined by GC, and results are displayed in A. 1. An Agilent 19091N–133 GC equipped with a flame ionization detector and an autosampler was used to analyze fatty acid composition of vegetable oils. Peak separation was performed on a HP–INNOWAX capillary column (30 m × 0.25 mm × 0.25 μm) from Agilent Technologies. A 1.0 μL volume of each methyl–esterified sample with the internal standard was injected via a splitless injector at 250°C and a constant pressure of nitrogen with a column flow of 1.3 mL min\textsuperscript{-1}. Then, 1.0 μL of each methyl–esterified sample with the internal standard was injected at a 40:1 split ratio into the column with the following thermal profile: 200°C for 2 min, 200°C–240°C at 8.0°C min\textsuperscript{-1}, and 240°C for 15 min. The inlet and detector temperatures were set to 250°C. Fatty acid profile was determined by identifying and calculating relative peak areas. All samples were prepared and injected for three times.

2.4 Statistical analysis

The mean values were used to present the results. FTIR spectral data processing and statistical analysis were conducted using OMNIC 7.3 (Thermo Electron Inc., Madison, WI), TQ Analyst 7.2 (Thermo Electron Inc., Madison, WI). The figures were plotted using Origin Pro 9.0 (OriginLab, Northampton, MA). Classical multivariate procedures were applied to the spectral data. The methods include PCA, CA, and LDA using Minitab 16.2.3 (Minitab Inc. USA) and SPSS version 20.0 (IBM, Armonk, NY, USA).
The PLS method was used to establish a correlation calibration model between actual adulterated level and FTIR-predicted values. Using Spectral pretreatment methods, such as baseline correction, multiplicative scatter correction (MSC), spectral normalization vector (SNV), Norris derivative filter (NF), Savitzky-Golay filter (SF), first derivative, second derivative, etc., can increase data information and remove the spectral defects, such as base line shifting and peak overlapping. The performance of FTIR–PLS models was evaluated by the root mean standard error of cross-validation (RMSECV), root mean error of calibration (RMSEC), root mean error of prediction (RMSEP), and R². The predicted residual error sum of squares (PRESS) was used to select the optimal number of principal components. The calibration with the highest R² value and the lowest RMSEC and RMSEP was considered practical for adulteration analysis.

### 3 Results and Discussion

#### 3.1 FTIR spectra of EUSO

3.1.1 FTIR spectral differences of EUSO from other vegetable oils

Infrared spectra of vegetable oils show fairly similar infrared spectra given that certain absorption bands attributed to specific stretching and the same functional groups. The FTIR spectra reflect differences according to the various substitution patterns in oils, as well as in the chain length of their acyl moieties, unsaturation degree, and position. Spectra were obtained by a path length calibration equation that relates the effective path length to the absorbance at 4334 cm⁻¹.

Figure 1 exhibits the FTIR spectra and spectral differences of the tested vegetable oils. Two spectral regions, 1429–1377 cm⁻¹ and 1128–1110 cm⁻¹, of EUSO differed from that of other vegetable oils, as shown in Fig. 1(b) and (c). Compared with infrared spectra of adulterated oil samples, the spectra of EUSO were also distinguished from the two regions. The difference at 1429–1377 cm⁻¹ is attributed to –C–H stretching and =C–H bending while that at 1128–1110 cm⁻¹ is ascribed to –C–O stretching. This result illustrates that the varieties and contents of such functional groups in EUSO are distinguishable from other oils. Thus, the trace components and of fatty acid composition of EUSO (A. 1) are different from various oils.

3.1.2 Chemometric tools applied for EUSO differentiation based on FTIR spectra

The spectral characteristic peaks for authentication can be analyzed by classification calibration based on spectral differences. In this case, the regional differences of 1429–1377 cm⁻¹ and 1128–1110 cm⁻¹ were analyzed by PCA, CA, and LDA.

PCA is a projection method for multidimensional data and it defines the number of principal components (PC) by reducing the dimensionality of numerical data sets without much loss of information; the two first PCs obtained by reducing the dimensions are used to explain the maximum variation in a set of observations of possibly correlated
variables\(^{10,24}\). Visualization of infrared characteristic based on two FTIR regions of EUSO and adulterated samples is presented in Fig. 2. The dataset of FTIR spectra was transformed into a 2D PCA plot using the first two principal components PC1 (90\%) and PC2 (7.3\%) for 1429–1377 cm\(^{-1}\), and PC1 (98.3\%) and PC2 (1.5\%) for 1128–1110 cm\(^{-1}\). There was no overlapping between EUSO and other oils, and the total contribution variance of PC1 and PC2 were greater than 90\%. Thus, the FTIR spectra convey appropriate information for the classification task, and the first two PCs are able to explain the total variance of the FTIR dataset\(^{25,26}\).

As shown in Fig. 2(a), the seven vegetable oils and adulterated samples are mainly divided into three groups. The classification of the eight different varieties of vegetable oils is as follows: EUSO makes up one group, the adulterated oils make up the other groups, and the six vegetable oils make up another group. The group of adulterated oils and that of six vegetable oils were not close to that of EUSO. The calibrated results presented that all oil samples were classified properly by PE–FTIR method.

LDA is a class-modeling technique for dimensionality re-

---

**Fig. 2** PCA (a), LDA (b) and CA (c) score plots of oil samples at 1429-1377 cm\(^{-1}\) and 1128-1110 cm\(^{-1}\), respectively.
duction that maximizes the variance between categories and minimizes the variance within categories. The classification procedure is considered to have normal distribution and equal dispersion (covariance matrix). LDA was employed to differentiate adulteration based on the FTIR spectra of EUSO and other oil samples. The dataset of FTIR spectra is shown in a 3D-LDA plot (Fig. 2(b)). The linear discriminant (LDs) factors of both regions accounted for 99.7% (i.e., >85%). The LDA plots also show that various oils were divided into three classified clusters, and the EUSO and six vegetable oils were well clustered from the adulterated oils.

CA is a method of centering the cluster and grouping them in a specific cluster based on the distance from individual data. It can be applied to depict differences among a variety of vegetable oils. The between-group linkage method with a metric of squared Euclidean distance was used for categorizing the oil samples. The distances between different oil samples is presented as a dendrogram, where the horizontal axis represents the Euclidean distance among groups and the vertical axis indicates similarities between the different varieties of vegetable oils in terms of each of their attributes. Figure 2(c) presents the CA score plot of all the oil samples. Oil samples have different detailed information from spectral data, as dissimilarity was reflected in different clusters. They were obviously divided into two groups at Euclidean distance of 10 for 1429–1377 cm⁻¹ and 15 for 1128–1110 cm⁻¹. EUSO makes up one group, the adulteration oils and six pure vegetable oils were gathered in another group, which are the same as those in the PCA and LDA plot. All oil samples were perfectly classified in CA.

The obtained results indicate that EUSO can be authenticated based on FTIR spectra from the two regions (1429–1377 cm⁻¹ and 1128–1110 cm⁻¹).

3.2 SyFS spectra of EUSO

3.2.1 SyFS spectral differences of EUSO form other vegetable oils

SyFS is a useful tool for adulteration analysis because it can reflect differences in quantity and variety of fluorescence chemicals, such as tocopherols, sterols, polar compounds, and chlorophyll.

In vegetable oils, tocopherols are major fluorescence compounds, which leads to very high fluorescence intensity at 250–500 nm, as shown in Fig. 3(a). Although diluting samples in n-hexane can reduce inner filter effect, the SyFS spectra of all used vegetable oils are shown in the same shape. According to Fig. 3(b), a variety of peaks observed in SyFS spectra of EUSO can serve more precise determination of EUSO.

The SyFS spectra of EUSO and the other seven oils are shown in Fig. 3(c). SyFS spectra of oil samples were collected in the excitation wavelength region 250–700 nm at Δλ = 60 nm; (a) SyFS spectra of EUSO diluted in n-hexane at 1% (b) SyFS spectra of EUSO without treatment; (c) SyFS spectra of EUSO and other edible oils without treatment.
interval $\Delta\lambda = 60$ nm. The spectra of these oils show differences from each other. The absorbance intensity of spectra of these oils varied from 300–500 nm, indicating that these oils contain different variety and quantity of fluorescence chemicals. However, these fluorescent components mainly resulted in the spectral region 300–500 nm, especially the spectrum of EUSO, which presents several divided peaks. The fluorescence absorption peak in the region 600–700 nm occurred highly to EUSO. The positions of fluorescence absorption peaks are at the same region, but obvious differences in the fluorescence peak intensity are observed.

As presented in Fig. 3(c), the spectra of seven oils at 300–500 nm are different, which can be attributed to the tocopherols and phenols, and the low intensity band in the emission wavelength region of 600–700 nm is ascribed to the chlorophyll and other pigments\textsuperscript{16, 28}. Additional peaks observed from fluorescence spectra of EUSO at 438 nm and 468 nm are perhaps associated with heterocyclic compounds produced during processing, such as pyridines and furans\textsuperscript{16, 26}, and additional peaks at 517 nm, 613 nm, and 662 nm are attributed to chlorophyll and other pigments\textsuperscript{29} (Fig. 3(b)). The fluorescence spectral differences of EUSO and other six vegetable oils illustrated that EUSO was different from other oils in composition and content of fluorescent chemicals due to different raw materials and extraction technology during processing. Therefore, these observations from SyFS can be used to distinguish EUSO from other oils for qualitative and quantitative analysis.

3.2.2 Chemometric techniques applied for EUSO differentiation

Major differences of SyFS spectra between EUSO and other vegetable oils were observed in the wavelength regions 300–500 nm and 600–700 nm. Figure 4 shows the SyFS spectra of pure EUSO and EUSO adulterated with peanut oil at different levels: 0, 0.5, 1, 2.5, 5, 10, 20, 30, 40, and 50\% (w/w) in the wavelength region 250–700 nm. Higher intensities were observed in the spectra of adulterated samples from 300–500 nm and lower at 600–700 nm. As adulterated level increases, the differences of intensity also increase.

Interestingly, the same trend was observed in the spectra of EUSO adulterated with other vegetable oils, which may be the fluorescence masking effect from the fluorescence compounds in EUSO. Strong correlation between the EUSO adulteration level and the fluorescence intensities of...
Authentication of Eucommia ulmoides Seed Oil

the different regions of SyFS spectra was found, which preliminarily proved the feasibility of the method. Therefore, these SyFS regions were used to evaluate the quantitative adulteration of EUSO.

SyFS spectra of 60 adulterated oil samples and EUSO samples were analyzed by PCA plot based on different wavelength ranges. The first two PCs explain 85.1 % (Fig. 5a), 99.4 % (Fig. 5b), and 94.9 % (Fig. 5c) of the data matrix variance, as indicated in Fig. 5.

According to the PCA plots, the EUSO samples are clustered from other vegetable oils and adulterated oil samples. Thus, these fluorescence groups can be used to distinguish EUSO adulteration. This screening technique presented good agreement with the results of FTIR method.

3.3 Quantitative calibration of EUSO adulteration by FTIR and SyFS

PCA, LDA, and CA results were satisfactory for separating EUSO samples from adulterated oils and other vegetable oils. The FTIR and SyFS spectra were collected and the quantitative calibrations related to adulteration were analyzed.

3.3.1 Calibration optimization

To eliminate the sample inequality and the spectral fluctuation error caused by some interference such as high frequency random noise and baseline drift, data selection and different spectra preprocessing methods were developed to increase prediction accuracy during the quantitative analysis modeling. Original spectral data were preprocessed by baseline correction, MSC, NF, SF, first derivative, second derivative and SNV. Effects of different preprocessing methods and data selection on prediction accuracy and precision were analyzed.

PLS regression calibrations were developed in effective characteristic bands. PRESS values are an indicator of the correlation of calibration data and are used to specify the optimal number of factors. The best model includes the fewest number of factors such that the PRESS for that model is not significantly greater than the minimum PRESS value [30]. The accuracy of the results corresponds to degree of agreement between actual and predicted values using the PLS model upon RMSEC, RMSEP, and RMSECV [31].

Table 1 shows that the SNV method achieves the best effects, manifested by maximum R value (0.9868) and minimum RMSEC value (2.83). Therefore, the SNV was considered as optimal preprocessing method. The characteristic bands screened by the interval PLS algorithm contained spectral intervals, which still contain wavelength variables unrelated to the absorption of infrared spectra of the EUSO adulteration. The wide selection of spectral region reduces the proportion of the valid information; on the contrary, the narrow selection of spectral region may have omitted valid information. Therefore, the spectral region should be comprehensively screened and optimized.

Table 2 shows that the wavenumbers from 1118–1110 cm⁻¹ and 1429–1377 cm⁻¹ were used for EUSO adulteration. Thus, high R value (0.9931, 0.9937) and low RMSEP (2.75, 2.73), and RMSECV (2.81, 2.80) were acceptable.

3.3.2 Quantitative calibration of EUSO adulteration by FTIR

As shown in Fig. 6, two PLS calibration curves were employed to determine the relationship between actual adulteration level (%, w/w) and predicted adulteration level (%, w/w) at regions of 1429–1377 cm⁻¹ (a) and 1128–1110 cm⁻¹ (b). FTIR spectra without any other derivatives and smoothing processing gave the highest R² value (0.9875, 0.9863) and the lowest errors either in calibration (2.73, 2.75) or in prediction (2.00, 1.99). Furthermore, the

| Spectral preprocessing methods          | R     | RMSEC | RMSEP |
|----------------------------------------|-------|-------|-------|
| Original spectrum                      | 0.9773| 3.69  | 4.70  |
| First derivative                       | 0.9694| 4.27  | 4.79  |
| SNV                                    | 0.9868| 2.83  | 2.55  |
| SNV+First derivative                  | 0.9791| 3.54  | 3.86  |
| SNV+NF+ First derivative              | 0.9851| 3.00  | 2.68  |
| SF                                     | 0.9745| 3.90  | 5.26  |
| NF                                     | 0.9724| 4.06  | 4.22  |
| MSC                                    | 0.9794| 3.51  | 3.77  |
| SNV+SF+ First derivative              | 0.9798| 3.48  | 3.30  |
| Second derivative                      | 0.9072| 7.32  | 8.42  |
| SF+ First derivative                  | 0.9712| 4.15  | 4.91  |
| SNV+Second derivative                 | 0.9173| 6.93  | 7.92  |

Table 1 Results of calibrations with different preprocessing methods for EUSO adulteration.
number of PCs was 4. Quantitative models were established by FTIR spectra with the SNV treatments.

### 3.3.3 Quantitative calibration of EUSO adulteration by SyFS

Based on the finding that the SyFS spectra of EUSO obtained from the different adulterated amount of other vegetable oils are correlated with the peak intensities in the specific regions. Therefore, calibration of SyFS spectra was established between areas of the absorbance peak at different wavelength ranges and adulterated levels. Calibration plots of adulteration levels vs. absorbance measured at 600–700 nm (c) and 300–500 nm (d) are shown in [Fig. 6](#).

The two following regression equations with $R^2$ of 0.9875 and 0.9863 indicate that the actual and predicted adulteration levels are strongly and linearly related with a slope and correlation coefficient close to 1 ([Fig. 6](#)(a,b)). In general, the results indicated that the two FTIR–PLS models can be used to predict the adulterant content of samples. [Figures 6](#)(c) and (d) show a good linear relationship between peak area of fluorescence spectra ranged from 600–700 nm and 300–500 nm and different adulteration levels, with $R^2$ of 0.9900 and 0.9914, respectively.

### Table 2 Results of calibrations with different band selections for EUSO adulteration.

| Spectral region | PCs | PRESS | R    | RMSEP | RMSECV |
|-----------------|-----|-------|------|-------|--------|
| 4000–400 cm$^{-1}$ | 10  | 4161  | 0.9773 | 4.70  | 3.69   |
| 4000–3250 cm$^{-1}$ | 9   | 2251  | 0.9841 | 2.97  | 3.10   |
| 1118–1110 cm$^{-1}$ | 7   | 2135  | 0.9931 | 2.75  | 2.81   |
| 1429–1377 cm$^{-1}$ | 7   | 2107  | 0.9937 | 2.73  | 2.80   |
| 1500–600 cm$^{-1}$ | 7   | 2155  | 0.9846 | 2.81  | 3.04   |
| 3250–600 cm$^{-1}$ | 8   | 2057  | 0.9857 | 2.86  | 2.93   |
| 2523–1000 cm$^{-1}$ | 9   | 2312  | 0.9860 | 2.82  | 3.01   |
Authentication of Eucommia ulmoides Seed Oil

3.4 Sample validation of the calibrations based on both techniques

The validation set from 26 different adulteration oils was used to validate the performance of the proposed method. Adulterated oils were prepared in random adulterated amounts (0–50%). Validation was conducted to examine the discriminatory calibration based on FTIR and SyFS spectra.

As shown in Table 3, all samples were classified by the PCA and LDA using FTR spectra from 1128–1110 cm\(^{-1}\) and 1429–1377 cm\(^{-1}\). Applying PCA achieved a recognition rate at 100% at both regions of the FTIR spectra. While using LDA, only one sample was not identified, based on the FTIR region 1128–1110 cm\(^{-1}\) with recognition rate of 96%. However, in the region of 1429–1377 cm\(^{-1}\), 100% recognition was achieved. Using PCA, all 26 samples were detected based on SyFS regions at 250–700 nm and 600–700 nm with recognition rates of 100%, only two samples were not identified, based on the SyFS region at 350–700 nm with recognition rate of 92%. The non-detectable samples were those that were added with 0.45% adulterants.

For validation, plots of predicted value versus actual concentration of adulterants in EUSO at ranges of 1429–1377 cm\(^{-1}\), 1128–1110 cm\(^{-1}\), 600–700 nm, and 300–500 nm were developed. Good correlation was observed between the predicted and actual levels of adulteration. In the regions of FTIR and SyFS spectra, unknown concentration of adulterated oils in EUSO could be easily measured using the regression equation.

A simple distribution of scatter plots between the actual value and predicted value was obtained, as shown in Fig. 7, which indicated that the plots of actual versus predicted values exhibited slopes close to 1, intercepts close to 0, and \(R^2\) values higher than 0.9700, showing the good performance of the quantitative models. The RMSECV values of FTIR-PLS validation models were 2.20 and 2.23, respectively. The detection limits of the FTIR and SyFS methods were calculated by the triple standard deviation which was obtained when the blank sample, the sample adulterated with other vegetable oils at adulteration level of 0%, was detected more than 20 times\(^{37}\). The detection limits of the both regions were 1% by FTIR. The relative sensitivities of the proposed models to determine the adulterated ratios were approximately 1% and 0.48% when using fluorescence spectra at 600–700 nm and 300–500 nm, respectively.

3.5 Discussion

FTIR and SyFS techniques were applied to EUSO to analyze the functional groups and fluorescence compounds. PE–FTIR was explored for accurate analyses of EUSO adulteration in conjunction with PCA, LDA, and CA by the absorbance band at the 1429–1377 cm\(^{-1}\), attributed to \(-\text{C–H stretching} and \ -\text{C–H bending}\), and 1128–1110 cm\(^{-1}\) ascribed to \(-\text{C–O stretching}\). SyFS, combined with PCA and chemometric multivariate regression analysis, was applied to analyze EUSO adulterated by blending six vegetable oils. The proposed methods provided valuable information for qualitative and quantitative analysis of EUSO adulterated with other vegetable oils.

The conventional ATR-FTIR methods provide weak signals due to short path length of ATR cell\(^{38,33,34}\). By the PE–FTIR procedure, spectra of the oil films were collected using a PE film as background spectrum, and effective path lengths of oil film spectra were normalized to a fixed path length of 0.15 mm by a path length calibration equation attributed to CH combination band\(^{36}\). Therefore, the PE–FTIR method is a feasible alternative for accurate analyses of EUSO adulteration because of simple procedure using a disposable PE film as a spectral acquisition accessory. The proposed method in the present study exhibited a very low detection limit when compared with previously reported techniques, e.g. ATR-FTIR combined with chemometrics (5%)\(^{36}\), mid-infrared spectroscopy (10%)\(^{37}\), and near-infrared spectrometry (3%)\(^{38}\). The proposed method can

| Methods | Regions               | No. of samples | No. of correct predictions | Recognition rate (%) |
|---------|-----------------------|----------------|----------------------------|----------------------|
| FTIR    | 1128–1110 cm\(^{-1}\) | 26             | 26                         | 100                  |
|         | 1429–1377 cm\(^{-1}\) | 26             | 26                         | 100                  |
| LDA     | 1128–1110 cm\(^{-1}\) | 26             | 25                         | 96                   |
|         | 1429–1377 cm\(^{-1}\) | 26             | 26                         | 100                  |
| SyFS    | 600–700 nm            | 26             | 26                         | 100                  |
|         | 300–500 nm            | 26             | 24                         | 92                   |
classify and authenticate EUSO with a detection limit of 0.48%, and the determining limit of the proposed quantitative calibrations is 1%. Both calibrations and validations presented a good recognition rates, indicating that the PE based FTIR method is more reproducible and slightly more sensitive.

Three different wavelength regions, namely, 250–700 nm, 300–500 nm, and 600–700 nm, were used for authentication EUSO coupled with PCA and chemometric multivariate regression analysis based on fluorescence detection. The quantitative adulteration of oil was investigated based on fluorescence spectroscopy combined with multivariate data analysis\(^{39, 40}\), in which the sample preparation in these studies was complicated and the detected limit (8.9%) was higher than that in our study. In contrast with these studies, the oil sample preparation of the proposed SyFS method is very simple, i.e., no need of dilution of oil samples in n-hexane. Therefore, sensitivity was improved and the relative sensitivity for the determination of adulterated levels was 1% and 0.48% based on SyFS spectra regions of 600–700 nm and 300–500 nm, respectively. Under the optimum condition, quantitative regression analysis presented high linearity and the method could detect as low as 0.48% of other oils adulterated in EUSO. Therefore, the proposed SyFS method is practical and accurate for the detection of adulterated oils. The qualitative and quantitative analysis methods to authenticate EUSO from other oils by PE–FTIR and SyFS were compared. The PE–FTIR technique is faster than the SyFS technique given that its accessory does not require a cleaning procedure. Moreover, the SyFS provides better prediction results and lower detection limit than PE–FTIR spectroscopy in blind sample validation sets. Overall, both PE–FTIR and SyFS are capable for EUSO adulteration.

### Conclusion

This study employed FTIR and SyFS spectroscopic methods for the authentication of adulterated EUSO and six different vegetable oils. Differences in the FTIR spectra of the oil samples indicated variations in fatty acid composition and other parameters. Therefore, differentiation of adulterants was achieved by using the bands at 1429–1377 cm\(^{-1}\) and 1128–1110 cm\(^{-1}\) in combination with PCA, LDA, and CA. The recognition rates of calibration and validation model were 100%. The fluorescence spectral differences between EUSO and the other six vegetable oils can be attributed to the different kinds or different amounts of fluorescent compounds. Analysis showed that fluorescence
spectral differences at 600–700 nm were related to the content of chlorophyll and other pigments compounds, whereas differences at 300–500 nm were mainly attributed to tocopherol and phenol contents. The calibration and validation model of PCA plot in the wavelength region 250–700 nm, 600–700 nm, and 300–500 nm recognition rates were desirable. The results of present study indicated that FTIR and SyFS can be used for qualitative and quantitative detection of EUSO adulteration.

Acknowledgements
This work was supported by the National Natural Science Foundation of China (NO: 31671819).

Compliance with Ethical Standards
Funding: Funding was provided by National Natural Science Foundation of China (No. 31671819).
Conflict of Interest: Keqing Hu, Zongyao Huyan, Syed Tufail Hussain Sherazi and Xiuzhu Yu declare that they have no conflict of interest.
Ethical Approval: This article does not contain any sections that require ethical approval.

Supporting Information
This material is available free of charge via the Internet at http://dx.doi.org/jos.68.10.5650/jos.ess19160

References
1) Liu, H.; Li, K.; Zhao, J.; Deng, W. Effects of polyphenolic extract from Eucommia ulmoides Oliver leaf on growth performance, digestibility, rumen fermentation and antioxidant status of fattening lambs. Anim. Sci. J. 89, 888-894 (2018).
2) Bai, M.M.; Shi, W.; Tian, J.M.; Lei, M.; Kim, J.H.; Sun, Y.N.; Kim, Y.H.; Gao, J.M. Soluble epoxide hydrolase inhibitory and anti-inflammatory components from the leaves of Eucommia ulmoides Oliver (duzhong). J. Agri. Food Chem. 63, 2198-205 (2015).
3) He, X.; Wang, J.; Li, M.; Hao, D.; Yang, Y.; Zhang, C.; He, R.; Tao, R. Eucommia ulmoides Oliv.: ethnopharmacology, phytochemistry and pharmacology of an important traditional Chinese medicine. J. Ethnopharmacol. 151, 78-92 (2014).
4) Tajik, N.; Tajik, M.; Mack, I.; Enck, P. The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: a comprehensive review of the literature. Nutr. Res. Rev. 56, 2215-2244 (2017).
5) Hirata, T.; Kobayashi, T.; Wada, A.; Ueda, T.; Fujikawa, T.; Miyashita, H.; Ikeda, T.; Tsukamoto, S.; Nohara, T. Anti-obesity compounds in green leaves of Eucommia ulmoides. Bioorg. Med. Chem. Lett. 21, 1786-1791 (2011).
6) Yen, G.-C.; Hsieh, C.-L. Antioxidant activity of extracts from Du-zhong(Eucommia ulmoides) toward various lipid peroxidation models in vitro. J. Agri. Food Chem. 46, 3952-3957 (1998).
7) Zhang, L.-X.; Ji, X.-Y.; Tan, B.-B.; Liang, Y.-Z.; Liang, N.-N.; Wang, X.-L.; Dai, H. Identification of the composition of fatty acids in Eucommia ulmoides seed oil by fraction chain length and mass spectrometry. Food Chem. 121, 815-819 (2010).
8) Yang, R.N.; Zhang, L.X.; Li, P.W.; Yu, L.; Mao, J.; Wang, X.P.; Zhang, Q. A review of chemical composition and nutritional properties of minor vegetable oils in China. Trends Food Sci. Tech. 74, 26-32 (2018).
9) Rodriguez-Saona, L.E.; Allendorf, M.E. Use of FTIR for rapid authentication and detection of adulteration of food. Annu. Rev. Food Sci. T. 2, 467-483 (2011).
10) Esteki, M.; Simal-Gandara, J.; Shahsavari, Z.; Zandbaaf, S.; Dashtaki, E.; Vander Heyden, Y. A review on the application of chromatographic methods, coupled to chemometrics, for food authentication. Food Control 93, 165-182 (2018).
11) Wernig, F.; Buegger, F.; Pritsch, K.; Splivallo, R. Composition and authentication of commercial and homemade white truffle-flavored oils. Food Control 87, 9-16 (2018).
12) Huyan, Z.Y.; Ding, S.X.; Liu, X.L.; Yu, X.Z. Authentication and adulteration detection of peanut oils of three flavor types using synchronous fluorescence spectroscopy. Anal. Methods 10, 3207-3214 (2018).
13) Lee, J.Y.; Park, J.H.; Mun, H.; Shim, W.B.; Lim, S.H.; Kim, M.G. Quantitative analysis of lard in animal fat mixture using visible Raman spectroscopy. Food Chem. 254, 109-114 (2018).
14) Xu, L.; Fei, T.; Li, Q.; Yu, X.; Liu, L. Qualitative analysis of edible oil oxidation by FTIR spectroscopy using a mesh "cell". Anal. Method. 7, 4328-4333 (2015).
15) Guillén, M.D.; Cabo, N. Infrared spectroscopy in the study of edible oils and fats. J. Sci. Food Agric. 75, 11-11 (1997).
16) Sikorska, E.; Górecki, T.; Khmelinskii, I.V.; Sikorski, M.; Kozioł, J. Classification of edible oils using synchronous scanning fluorescence spectroscopy. Food Chem. 89, 217-225 (2005).
17) Ge, F.; Chen, C.; Liu, D.; Zhao, S. Rapid quantitative determination of walnut oil adulteration with sunflower oil using fluorescence spectroscopy. Food Anal. Method. 7, 146-150 (2013).
18) Rohman, A.; Che Man, Y.B.; Hashim, P.; Ismail, A. FTIR spectroscopy combined with chemometrics for analysis of lard adulteration in some vegetable oils. *CyTA-J. Food* 9, 96-101 (2011).

19) Huyan, Z.; Ding, S.; Liu, X.; Yu, X. Authentication and adulteration detection of peanut oils of three flavor types using synchronous fluorescence spectroscopy. *Anal. Methods* 10, 3207-3214 (2018).

20) Chen, X.; Yu, X.; Wang, Y.; Yang, Y.; Zhang, J. Determination of polar components in frying oils by Fourier-transform near-infrared spectroscopy. *J. Oleo Sci.* 64, 255-61 (2015).

21) Chen, X.; Yu, X.; Wang, Y.; Yang, Y.; Zhang, J. Determination of polar components in frying oils by Fourier-transform near-infrared spectroscopy. *J. Oleo Sci.* 64, 255-61 (2015).

22) Maggio, R.M.; Cerretani, L.; Chiavaro, E.; Kaufman, T.S.; Bendini, A. A novel chemometric strategy for the estimation of extra virgin olive oil adulteration with edible oils. *Food Control* 21, 890-895 (2010).

23) Zhang, Q.; Liu, C.; Sun, Z.; Hu, X.; Shen, Q.; Wu, J. Authentication of edible vegetable oils adulterated with used frying oil by Fourier transform infrared spectroscopy. *Food Chem.* 132, 1607-1613 (2012).

24) Rodriguez-Campos, J.; Escalona-Buendia, H.B.; Orozco-Avilla, I.; Lugo-Cervantes, E.; Jaramillo-Flores, M.E. Dynamics of volatile and non-volatile compounds in cocoa (*Theobroma cacao* L.) during fermentation and drying processes using principal components analysis. *Food Res. Int.* 44, 250-258 (2011).

25) Hong, X.; Wang, J.; Qi, G. E-nose combined with chemometrics to trace tomato-juice quality. *J. Food Eng.* 149, 38-43 (2015).

26) Li, B.; Wang, H.; Zhao, Q.; Ouyang, J.; Wu, Y. Rapid detection of authenticity and adulteration of walnut oil by FTIR and fluorescence spectroscopy: a comparative study. *Food Chem.* 181, 25-30 (2015).

27) Hai, Z.; Wang, J. Detection of adulteration in camellia seed oil and sesame oil using an electronic nose. *Eur. J. Lipid Sci. Technol.* 108, 116-124 (2006).

28) Dupuy, N.; Le Drea, Y.; Ollivier, D.; Artaud, J.; Pinatel, C.; Kister, J. Origin of French virgin olive oil registered designation of origins predicted by chemometric analysis of synchronous excitation-emission fluorescence spectra. *J. Agric. Food Chem.* 53, 9361-9368 (2005).

29) Tan, J.; Li, R.; Jiang, Z.T.; Tung, S.H.; Wang, Y.; Shi, M.; Xiao, Y.Q.; Jia, B.; Lu, T.X.; Wang, H. Synchronous front-face fluorescence spectroscopy for authentication of the adulteration of edible vegetable oil with refined used frying oil. *Food Chem.* 217, 274-280 (2017).

30) Kovalenko, I.V.; Rippke, G.R.; Hurburgh, C.R. Measurement of soybean fatty acids by near-infrared spectroscopy: Linear and nonlinear calibration methods. *J. Am. Oil Chem. Soc.* 83, 421-427 (2006).

31) De Souza, L.M.; Mitsutake, H.; Gontijo, L.C.; Borges Neto, W. Quantification of residual automotive lubricant oil as an adulterant in Brazilian S-10 diesel using MIR spectroscopy and PLS. *Fuel* 130, 257-262 (2014).

32) Kuselman, I.; Sherman, F. Assessment of limits of detection and quantitation using calculation of uncertainty in a new method for water determination. *Accreditation and Quality Assurance* 4, 124-128 (1999).

33) De Santana, F.B.; Mazivila, S.J.; Gontijo, L.C.; Neto, W.B.; Poppi, R.J. Rapid discrimination between authentic and adulterated andiroba oil using FTIR-HATR spectroscopy and random forest. *Food Anal. Method.* 11, 1927-1935 (2018).

34) Elzeey, B.; Pollard, D.; Fakayode, S.O. Determination of adulterated neem and flaxseed oil compositions by FTIR spectroscopy and multivariate regression analysis. *Food Control* 68, 303-309 (2016).

35) Dong, X.; Li, Q.; Sun, D.; Chen, X.; Yu, X. Direct FTIR analysis of free fatty acids in edible oils using disposable polyethylene films. *Food Anal. Method.* 8, 857-863 (2014).

36) Vasconcelos, M.; Coelho, L.; Barros, A.; de Almeida, J.M.M.M.; Yildiz, F. Study of adulteration of extra virgin olive oil with peanut oil using FTIR spectroscopy and chemometrics. *Cogent Food Agric.* 1, 1-13 (2015).

37) Gurdeniz, G.; Ozen, B. Detection of adulteration of extra-virgin olive oil by chemometric analysis of mid-infrared spectral data. *Food Chem.* 116, 519-525 (2009).

38) Zeng, L.L.; Song, Z.Q.; Zheng, X.; Tu, B.; Yin, C.; He, D.P.; Qi, P.S. NIR spectroscopy-based qualitative and quantitative detection of adulteration of peanut oil. *Appl. Mech. Mater.* 687-691, 795-801 (2014).

39) Dankowska, A.; Matecka, M. Application of synchronous fluorescence spectroscopy for determination of extra virgin olive oil adulteration. *Eur. J. Lipid Sci. Technol.* 111, 1233-1239 (2009).

40) Dankowska, A.; Malecka, M.; Kowalewski, W. Discrimination of edible olive oils by means of synchronous fluorescence spectroscopy with multivariate data analysis. *Grasas y Aceites* 64, 425-431 (2013).