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Feasibility study of quantifying and discriminating soybean oil adulteration in camellia oils by attenuated total reflectance MIR and fiber optic diffuse reflectance NIR

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Abstract

Camellia oil is often the target for adulteration or mislabeling in China because of it is a high priced product with high nutritional and medical values. In this study, the use of attenuated total reflectance infrared spectroscopy (MIR-ATR) and fiber optic diffuse reflectance near infrared spectroscopy (FODR-NIR) as rapid and cost-efficient classification and quantification techniques for the authentication of camellia oils have been preliminarily investigated. MIR spectra in the range of 4000–650 cm^{-1} and NIR spectra in the range of 10,000–4000 cm^{-1} were recorded for pure camellia oils and camellia oil samples adulterated with varying concentrations of soybean oil (5–25% adulterations in the weight of camellia oil). Identifications is successfully made base on the slightly difference in raw spectra in the MIR ranges of 1132–885 cm^{-1} and NIR ranges of 6200–5400 cm^{-1} between the pure camellia oil and those adulterated with soybean oil with soft independent modeling of class analogy (SIMCA) pattern recognition technique. Such differences reflect the compositional difference between the two oils with oleic acid being the main ingredient in camellia oil and linoleic acid in the soybean oil. Furthermore, a partial least squares (PLS) model was established to predict the concentration of the adulterant. Models constructed using first derivative by combination of standard normal variate (SNV), variance scaling (VS), mean centering (MC) and Norris derivative (ND) smoothing pretreatments yielded the best prediction results With MIR techniques. The *R* value for PLS model is 0.994.The root mean standard error of the calibration set (RMSEC) is 0.645, the root mean standard error of prediction set (RMSEP) and the root mean standard error of cross validation (RMSECV) are 0.667 and 0.85, respectively. While with NIR techniques, NIR data without derivative gave the best quantification results. The *R* value for NIR PLS model is 0.992. The RMSEC, RMSEP and RMSECV are 0.70, 1.78 and 1.79, respectively. Overall, either of the spectral method is easy to perform and expedient, avoiding problems associated with sample handling and pretreatment than the conventional technique.

Keywords: MIR; NIR; Camellia oil; Adulteration; Classification; Quantification

1. Introduction

Camellia oil, produced from the seed of camellia (Camellia Oleifera Abel) by oil mill or solvent extraction is one of the favorite cooking oil used wildly in Southern China and Southeast Asia. Camellia oil has much in common with olive oil in chemical composition, with high amounts of oleic acids and linoleic acid and often titled as ''Eastern Olive Oil''. The action of camellia oil is generally known to aid cholesterol loss and resistance to stress (Fu & Zhou, 2003). It demands higher price on the market than other plant oils on account

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of the nutritional and medical properties. For these reasons, just like olive oil, camellia oil is often the target for adulteration or substitution by other cheaper oils such as soybean oil.

Adulteration of commercial food products has been known to exist for a long time and various physical and chemical tests have been reported to address this problem (Lai, Kemsley, & Wilson, 1995). Among them, authenticity of olive oil and to detect the level in it has attracted much attention. Unfortunately, little attention was paid to the authenticity of camellia oils.

The methods to identify olive oil in general involve conventional method such as HPLC (Salivaras & Mccurdy, 1992), GC (Kapoulas & Passaloglou-Emmanouillidou, 1981), UV (Passaloglou-Emmanouillidou, 1990) and NMR (Sachhi, Addeo, & Paolillo, 1997), which is expensive, time-consuming or destructive. Mid-infrared spectra (MIR) scanned in the region of 4000–650 cm^{-1} consisting of fundamental and characteristic bands whose frequencies and intensities can be used to identify the relevant functional groups. The attenuated total reflectance (ATR) technique applied with no or minimal sample preparation and have been applied successfully to quantifying sunflower oil adulteration in extra virgin oil (Kupper, Heise, Lampen, Davies, & McIntyre, 2001; Lai et al., 1995) and classifying of edible fats and oils (Dupuy, Duponchel, Huvenne, Sombret, & Legrand, 1996). Near-infrared spectroscopy (NIR) lies between the visible and mid-infrared region (10,000–4000 cm^{-1}). Light absorption in this region is primarily due to the overtones and combinations of fundamental vibration bands occurring in the MIR region. Though NIR bands are often broad and overlapping, it has demonstrated a capability for discrimination between sets of similar biological materials such as coffee varieties (Downey, Robert, Bertrand, & Kelly, 1990), milk powder (Rannou & Downey, 1997), and Chinese herbs (Li, Tsang, & Lee, 2001). And also demonstrate the potential of discrimination between authentic extra virgin olive oil and the same oils adulterated by the addition of sunflower oil and quantifying the level in it (Downey, Mcintyre, & Davis, 2002). Furthermore, near-infrared with fiber optic diffuse reflectance probe can be applied with little sample preparation and can be controlled in the remote and made the whole procedure more convenient.

In this work, our object is to do a preliminary investigation to examine and compare the performance of attenuated total reflectance infrared spectroscopy (MIR-ATR) and fiber optic diffuse reflectance near infrared spectroscopy (FODR-NIR) in the identification of camellia oil adulteration using pattern recognition techniques including soft independent modeling of class analogy (SIMCA) and principle component analysis (PCA), and also in the quantification of camellia oil adulteration using partial least square (PLS) algorithm.

2. Material and methods

2.1. Sample preparation

Ten camellia oils and one soybean oil samples were purchased from local grocery stores. The authenticity of the samples were approved and permitted by the local governmental food regulatory agencies. The camellia oil and soybean oil samples were mixed together in accurately weighed proportions to obtain the calibration and validation sets of 50 adulteration samples. The amount of soybean oil as the adulterate in camellia oil ranged from 5% to 25% (w/w). There is no visible difference between the pure camellia oils and adulteration oils in their appearance to the naked eyes. The samples containing soybean oils were marked as adulteration oils while camellia oils were marked as pure camellia oils.

2.2. Instrumentation

2.2.1. MIR-ATR

The middle infrared spectra were acquired using a Nicolet 370 MIR spectrometer equipped with a DTG detector and flat zinc selenide single reflectance crystal using blunt thin glass rod as described in the reference (Christy, Egeberg, & Østensen, 2003). Each spectrum was recorded in the region of 4000–650 cm^{-1} by an average of 32 scans at a resolution of 4 cm^{-1}. Each sample was recorded three times and the corresponding average spectra were calculated and saved for the following uses. Between samples, the crystal was cleaned with dichloromethane, alcohol, distilled water and then purged by air to dryness. The background was collected before every sample was measured.

2.2.2. FODR-NIR

Near infrared spectra were recorded in the range of 4000–10,000 cm^{-1} at 8 cm^{-1} intervals using a Nicolet 360N NIR spectrometer equipped with a fiber optic diffuse reflectance probe. Fiber optic diffuse reflectance NIR is a good tool for measure opaque solid and liquid, and can do the same measurement to the transparent solid and liquid by putting a ceramic tile opposite to the probe as to let the light diffuse. In this experiment, all of the spectra were recorded as absorbance with respect to a smooth ceramic tile reference standard. NIR spectra were collected by immersing the fiber optic probe into each sample contained in a glass bottle, which was placed on the ceramic tile reference standard. The bottom of the bottle is clear and the wall is shield from ambient light during measurement. The distance between the optical fibers and the reflecting ceramic tile is 0.3 cm, resulting with an actual pathlength of 0.6 cm. Each spectrum was obtained by an average of 110 scans. Each sample was recorded three times and the corresponding average spectra were calculated and

saved for the following uses. Between samples, the fiber probe were washed with methyl alcohol and rinsed with distilled water and then purge to dry. The background spectra were collected by putting the probe in the empty glass bottle on top of the reference ceramic tile before every sample was measured.

2.2.3. Chemometrics

Chemometric analysis including classification and quantification analysis were carried out using TQ analyst 6.0 software package (Thermo-Nicolet).

Classification was performed by SIMCA and PCA techniques in the range of 1132–885 cm^{-1} for MIR and 6200–5400 cm^{-1} for NIR at the 95% confidence level.

Quantification of soybean oil adulteration levels was calculated by PLS regression analysis in the TQ analyst 6.0. The spectral regions used for discriminate analysis was also used for PLS models. The optimum number of PLS factors was determined by cross-validation employing cancellation one standard at a time by plotting the number of factors against the root mean square error of cross validation (RMSECV) and determining the minimum. The relative performance of the established model was accessed by the required number of factors, the root mean square error of calibration (RMSEC), and the RMSECV, and its predictive ability was evaluated from the root mean square error of prediction (RMSEP).

Several spectral pretreatments were tried to optimize the model. Mean Centering (MC) removes the common information from the spectra. Variance Scaling (VS) allows all spectra in a scale unit by dividing each data point in each calibration spectrum by its estimated standard deviation. Standard normal variate (SNV) scales the spectral data in order to compensate for differences in sample pathlength. Norris Derivative (ND) and Savitzky–Golay (SG) smoothing filters are useful for improving the appearance of peaks that are obscured by random noise. Furthermore, ND is often used to enhance a sharp band that is overlapped by another broad band.

3. Results and discussion

3.1. Spectra features of MIR

The spectral differences based on the chemistry were that oleic acid is the main component of camellia oil while the linoleic acid is the main component of the soybean oil (Table 1). Those spectral differences can be successfully utilized for qualitative classification. The typical ATR-MIR spectra of pure camellia oil and soybean oil are shown in Fig. 1. The spectra are offset to allow visual comparison. The assignments of prominent peaks include: C–H stretching mode in the wavenumber region of 2800–3100 cm^{-1}, C═O stretching in the region of 1700–1800 cm^{-1}, and C–O–C stretching and C–H bending in the region of 900–1400 cm^{-1} (Tay, Singh, Krishnan, & Gore, 2002). The entire range of spectra looks closely similar for the two oils to the naked eyes. This is due to the similar chemical composition of the oil pairs. However, if one examines the spectra closely, minor difference between adulteration and pure camellia oil samples are observed at several different spectra regions of around 912, 1097 and 1120 cm^{-1}, corresponding to CH banding vibration and CH deformation vibrations of fatty acid.

Table 1
Main components of olive oil, camellia oil and soybean oil (w/w) (Fu and Zhou, 2003; Xu, 1996)

	Olive oil	Camellia oil	Soybean oil
Oleic acid (%)	75.0	75.1	22.4
Linoleic acid (%)	9.0	10.5	53.9
Flax acid (%)	1.0	0.87	9.2
Stearic acid, Palm acid and others (%)	15	13.5	14.3

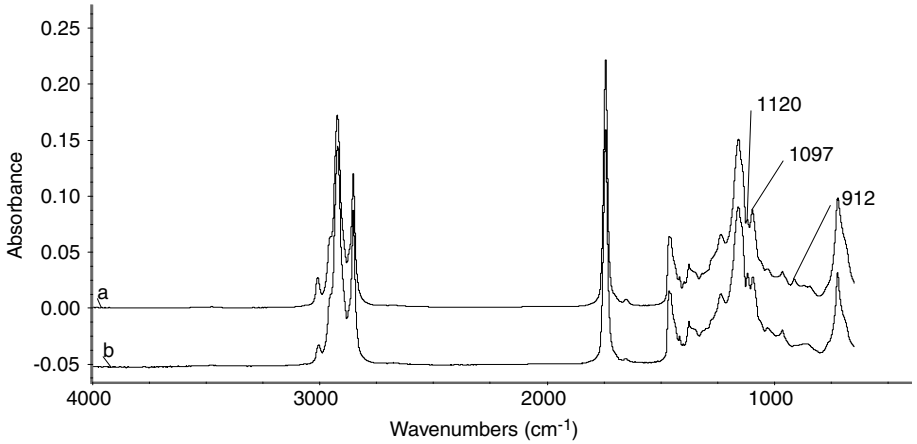

Fig. 1. The typical ATR-MIR spectra of pure camellia oil and soybean oil: (a) soybean oil; (b) camellia oil.

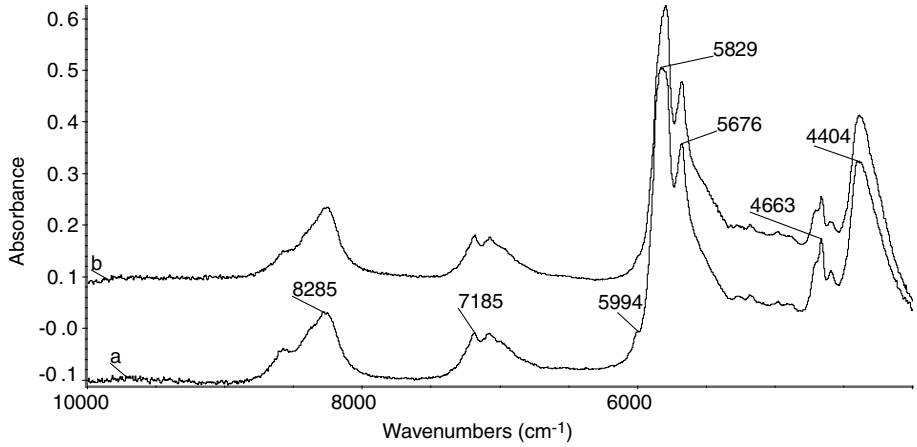

Fig. 2. The typical FODR- NIR spectra of pure camellia oil and soybean oil: (a) soybean oil; (b) camellia oil.

3.2. Spectra features of NIR

The typical FODR-NIR spectra of pure camellia oil and soybean oil are shown in Fig. 2. The spectra are offset to allow visual comparison. Absorption maxima are clearly observed at 8285, 7185, 5829, 5767, 4404 cm^{-1} together with small absorption bands at 4800–4540 cm^{-1}. Bands around 8285 cm^{-1} arise from the second overtones of CH stretching vibrations, while those at 5829, 5767 cm^{-1} are attributable to the first overtones of CH stretching vibrations of $–CH_3$, $–CH_2$ and –HC═CH–. Absorption at 4404 cm^{-1} arises from combination bands of CH stretching vibrations of $–CH_3$ and $–CH_2$. Fig. 2 shows that NIR spectra of both oils have no noticeable difference other than those around 5994 cm^{-1}, where soybean oil has a very weak peak while camellia shows no clear peaks. And the peak around 5829 cm^{-1} of soybean oil is slightly wider than that of pure camellia oil.

3.3. Classification

PCA was performed initially to extract information and examine the qualitative difference for all the samples with MIR spectra of 1132–885 cm^{-1} and with NIR spectra of 6200–5400 cm^{-1}, where the slightly differences appear. No substantial differences in the raw MIR and NIR spectra base on pure camellia oil and the adulteration oil were evident. PCA is a data reconstruction and reduction method, each principal component represents an independent source of spectral variation in the data. The differentiation between the pure camellia oils and adulteration oils is clear in the two-dimensional score plot using the first and the fourth principal component (PC) with MIR spectra and using the third and the fourth PC with NIR spectra (Fig. 3).

The model used to classify the pure camellia oils and the adulteration oils was constructed by using a supervised technique namely SIMCA. In SIMCA, a class is modeled by means of principle component. A separated PCA is calculated for each class. Each class is divided into a calibration set and a validation set at random as shown in Table 2. The division of samples was indented to assign roughly 70% of the total spectra into the calibration set. To enable comparison of the two spectral techniques the same sets of samples were used. Fisher's test is subsequently applied in order to estimate the likelihood of a sample belonging to the class defined by the spectra of the calibration set, and the validity of the model is tested on the validation set. Summary of the SIMCA model and their performance are described in Table 2. The table shows that pure camellia oils and the adulteration oils are classified correctly with both ATR-MIR and FODR-NIR techniques.

3.4. Quantification

Quantification of the soybean oil contents in the adulterated oil samples was performed using PLS algorithm. The spectral regions used for discriminate analysis was also used for PLS models.

For chemometric evaluation, the samples of all the adulteration oils were divided into a calibration and a validation set. To enable comparison of the spectral techniques the same sets of samples were used. The calibration set consisted of 35 samples while the validation set consisted of 15 samples. The division into sets was done in order to obtain similar mean values and standard deviations so that both sets spanned the full range of soybean oil contents (Muik, Lendl, & Molina-Diaz, 2004) (Table 3).

Several spectral pretreatments including SNV, VS, SG and ND, were investigated for optimization of the calibration model. The best calibration model was developed by "try" (Ren & Chen, 1999), which refers to evaluation the performance of calibration model when every combination of derivative treatments, spectral pretreatments, and statistical models were tested.

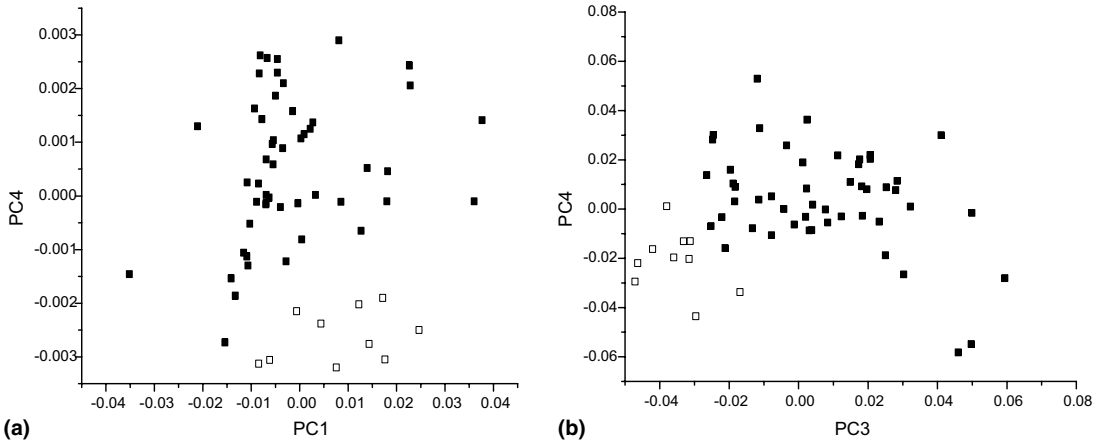

Fig. 3. The two-factor plot in principle components analysis for the camellia oil and adulteration oil using the first and fourth factor with MIR spectra and using the third and fourth factor with NIR spectra. □, pure camellia oils; ■, adulteration oils; (a) MIR spectra; (b) NIR spectra.

Table 2
SIMCA classification of pure camellia oils and the adulteration oils (at 95% confidence)

	Class	Calibration set	Validation set	Misclassified
ATR-MIR (raw date, 1132–885 cm^{-1})	Pure camellia oil	7	3	0
	Adulteration oil	35	15	0
FODR-NIR (raw date, 6200–5400 cm^{-1})	Pure camellia oil	7	3	0
	Adulteration oil	35	15	0

Table 3
Characteristics of calibration and validation sets for soybean oil content in adulteration oil

	Samples	Soybean oil content (%)	Mean	stdev[a]
Calibration set	35	4.67 ~ 24.67	13.46	5.78
Validation set	15	5.50–22.40	13.84	5.49

[a] Standard deviation.

For MIR spectra, a summary of results is shown in Table 4. From Table 4, we can see that models using 2nd derivative data led to high RMSEP and RMSECV values but relative low RMSEC values compared with raw and 1st derivative date, the results generally could be due to overfitting of the calibration. However, applying smoothing led to the model with enhanced performance in both raw and derivative data. ND is preferred than SG in the model. Over all, the results show that the model produce the best prediction equation is the one which combine SNV, VS, MC and ND smoothing pretreatments, respectively, in the range of 1132–885 cm^{-1} of 1st derivative MIR spectra.

For NIR spectra, a summary of results is shown in Table 5. From Table 5, we can see that raw spectra with no derivative process gave good results. SNV pretreatment alone is not enough to handle the NIR data and produce acceptable results. Several pretreatments couplings are needed. SNV coupled with VS, SG, MC, and ND can enhance the performance of the model significantly. In short, the results show that the model produce the most accurate prediction equation is the combination of SNV, VS, MC and SG pretreatments in the ranges of 6200–5400 cm^{-1} of NIR spectra.

A graphical display of the regressions produced using these models with the best performances are shown in Figs. 4 and 5 for MIR and NIR spectra, respectively.

PLS loading 1 and 2 are shown in Figs. 6 and 7 for ATR-MIR and FODR-NIR, respectively. Loading 1 and 2 account for 52.6% and 21.2% of the variance in ATR-MIR spectra data; While in FODR-NIR spectra data, loading 1 and 2 account for 76.6% and 11.9% of the variance. It's difficult to relate the wavelengths in these loadings plot to specific oil component.

Table 4
Calibration and validation results of MIR models using different spectral pretreatments technique

	Pretreatments	Factor	*R*	RMSECV	RMSEC	RMSEP
Raw spectra	SNV	5	0.995	1.31	0.567	0.783
	SNV + VS + MC	5	0.9955	1.22	0.542	0.815
	SNV + VS + MC + SG[a]	5	0.9947	1.17	0.588	0.761
First derivative	SNV	4	0.9884	1.57	0.865	1.33
	SNV + VS + MC	4	0.995	1.4	0.571	1.31
	SNV + VS + MC + ND[b]	**5**	**0.9936**	**0.85**	**0.645**	**0.667**
	SNV + VS + MC + SG	4	0.9912	1.27	0.754	1.40
Second derivative	SNV	4	0.9018	4.89	2.46	4.59
	SNV + VS + MC	3	0.9111	4.11	2.35	4.32
	SNV + VS + MC + ND	3	0.9904	1.07	0.787	0.982
	SNV + VS + MC + SG	4	0.9912	1.76	0.755	2.01

Results from models with the best performance are marked in bold.
SNV, standard normal variate; VS, vairance scaling; MC, mean centering; SG, Savitzky-Golay; ND, Norris derivative; SG[a] (7 data points, 3 polynomial orders), ND[b] (5 segment length, 2 gaps between segments); RMSECV, root mean of square error of cross validation; RMSEC, root mean of square error of calibration set; RMSEP, root mean of square error of prediction set; *R*, correlation coefficient.

Table 5
Calibration and validation results of NIR models using different spectral pretreatments

	Pretreatments	Factor	*R*	RMSECV	RMSEC	RMSEP
Raw spectra	SNV	10	0.9988	1.64	0.274	2.9
	SNV + VS + MC	10	0.9987	1.86	0.287	2.02
	SNV + VS + MC + SG[a]	**8**	**0.9924**	**1.79**	**0.701**	**1.78**
First derivative	SNV	3	0.5444	5.27	4.78	5.52
	SNV + VS + MC	2	0.4858	5.45	4.98	5.49
	SNV + VS + MC + ND[b]	12	0.998	2.04	0.363	2.06
	SNV + VS + MC + SG	16	0.9997	4.6	0.0456	2.94
Second derivative	SNV	2	0.1193	6.42	5.98	5.66
	SNV + VS + MC	1	0.107	5.9	5.66	5.34
	SNV + VS + MC + ND	9	0.9878	2.45	0.888	2.27
	SNV + VS + MC + SG	2	0.4902	5.5	4.96	5.85

Results from models with the best performance are marked in bold.
SNV, standard normal variate; VS, vairance scaling; MC, mean centering; SG, Savitzky–Golay; ND, Norris derivative; SG[a] (7 data points, 3 polynomial orders), ND[b] (5 segment length, 2 gaps between segments); RMSECV, root mean of square error of cross validation; RMSEC, root mean of square error of calibration set; RMSEP, root mean of square error of prediction set; *R*, correlation coefficient.

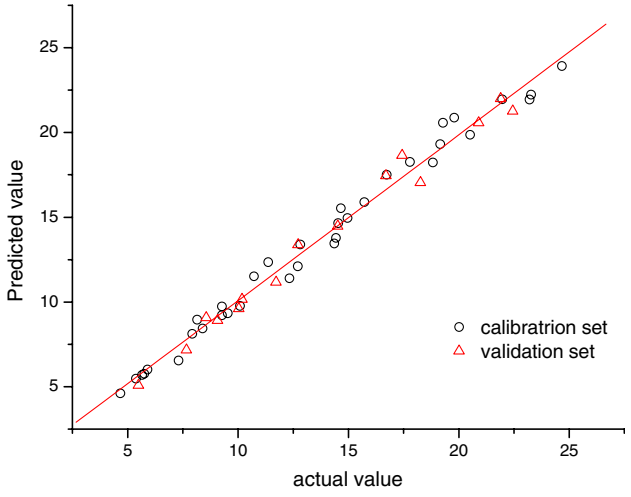

Fig. 4. PLS regression of predicted vs actual soybean oil content in pure camellia oil samples of ATR-MIR spectra ($n = 50$; 1132–885 cm^{-1} wavelength range).

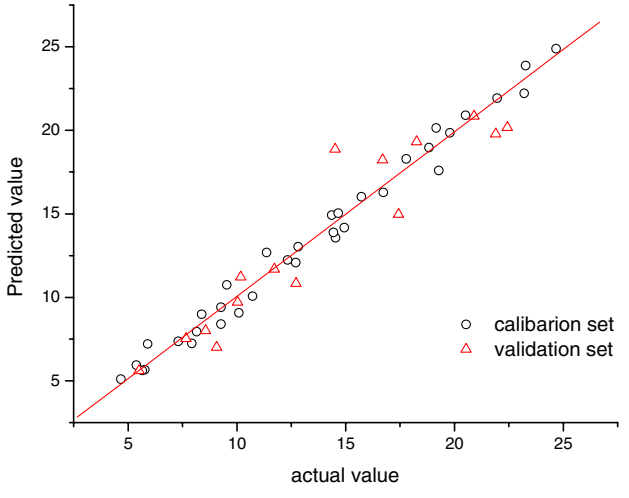

Fig. 5. PLS regression of predicted vs actual soybean oil content in pure camellia oil samples of FODR-NIR spectra ($n = 50$; 6200–5400 cm^{-1} wavelength range).

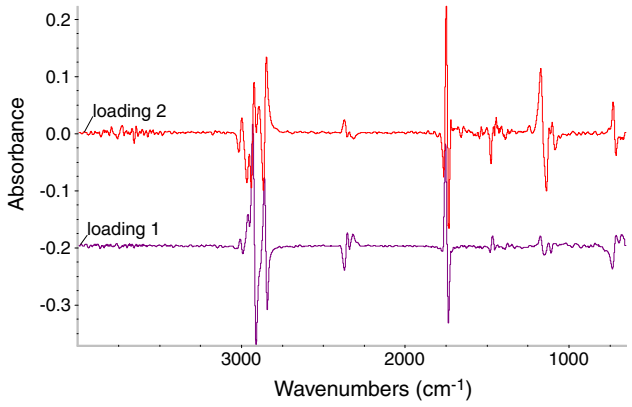

Fig. 6. PLS loadings 1 and 2 for ATR-MIR (soybean oil content prediction, 1st derivative data, SNV + VS + MC + ND pretreatment, offset for clarity).

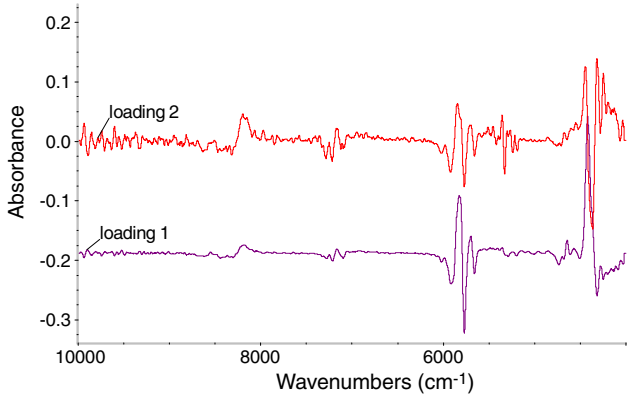

Fig. 7. PLS loadings 1 and 2 for FODR-NIR (soybean oil content prediction, SNV + VS + MC + ND pretreatment, offset for clarity).

4. Conclusions

This study give a preliminary investigation to examine and compare the performance of MIR-ATR and FODR-NIR spectrometry in the identification of authentic and adulterated oils and quantification the adulteration level in it using the same set of samples. According to the results presented it seems that both techniques should be able to classify the pure camellia oil and the same oils adulterated with soybean oils (5–25%, w/w) correctly with SIMCA algorithm at 95% confidence level. And quantification of the soybean oil adulterant was achieved by PLS regression with proper combinations of data pretreatments. Either of the spectral method is easy to perform and expedient, avoiding problems associated with sample handling and pretreatment than the conventional technique. It should be stressed that this work has only involved a single soybean oil and a limited number of camellia oils and therefore this work require extension to a great number of samples.

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