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Application of FTIR spectroscopy coupled with chemometrics for authentication of *Nigella sativa* seed oil

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Abstract. The present study is intended to analyze the presence of grape seed oil (GSO) in *Nigella sativa* L. seed oil (NSO) using Fourier transform infrared (FTIR) spectroscopy and gas chromatography (GC). FTIR spectroscopy coupled with multivariate calibration of partial least square can quantify the levels of GSO in NSO at wavelength number of 1114–1074, 1734–1382 and 3005–3030 cm^{-1}. The coefficient of correlation (R^2) obtained for the relationship between actual (x-axis) and FTIR predicted (y-axis) values are 0.981. The errors in cross validation and in prediction are 2.34% (v/v) and 2.37% (v/v), respectively.

Keywords: FTIR spectroscopy, grape seed oil, *Nigella sativa* L. seed oil, multivariate calibration

1. Introduction

Nigella sativa L. also known as black cumin has been consumed for centuries, especially in the Middle East and Southeast Asia. It has been widely distributed in Arab countries and other parts of the Mediterranean region [14]. This plant is one of the annual herbaceous plants that belong to *Ranunculaceae* family and widely known by various Arabic names such as '*Al-Habba Al-Sawdaa*' or '*Al-Kammoon Al-Aswad*', '*Habbet el Baraka*' and '*Shunez*' [2].

Nigella sativa L. seed oils (NSO) is one of the most extensively studied oils in recent years as a natural remedy for many ailments. NSO or extract has protective and curative actions. The extracts of NSO have been used as a natural remedy for asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness and influenza [1,22]. The seeds have been known to be carminative, stimulant, diuretic, emmenagogue and galactogogue, and used in treating fever [27]. In addition, they were used as a condiment in bread and other dishes [19]. The lipid oil from the seed of NSO is rich in linoleic and oleic acids which are believed to be good for human health [7].

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The chemical composition of NSO is very rich and diverse. It contains amino acids, proteins, carbohydrates, fixed and volatile oils [15]. The main active constituent of the volatile oil of the NSO is Thymoquinone. Besides, NSO has pleasant taste and specific odor. NSO oil is 10–15 times more expensive than other edible oils such as grape seed oil, walnut oil and soybean oil. This fact has led the unscrupulous market players to adulterate this oil with the cheaper oils to increase their economic profit. Therefore, the detection and quantification of NSO must be addressed to assure its quality and safety.

Various accurate and reliable methods such as chromatography as reviewed by [11], differential scanning calorimetry [8], electronic nose [18] and wet chemical methods have been continuously proposed and developed by researchers to detect the oil blended into the authentic oil in order to protect the consumers from economic loss and incorrectly labeled oils. Some of these methods are impractical and too laborious. Therefore, rapid and accurate analytical methods must be developed in order to detect and to quantify the oil adulterants. In this study, we developed FTIR spectroscopy combined with chemometrics analysis for such purpose.

FTIR spectroscopy has received great attention in quantitative analysis of fats and oils over the years due to the main advantage of easy sample preparation with reduced or no-sample pre-treatment steps [28]. Its application to analysis of edible fats and oils can be considered as "green analytical chemistry" because this technique reduces or eliminates solvents and chemical reagents used in analysis which might be hazardous to human health and environment [21]. This technique has been successfully used to analyze VCO in ternary mixture system with palm oil and olive oil [25], palm kernel oil [17], lard in cod liver oil [24] and lard in animal fats [5].

Nowadays, the progress in computer technology and multivariate chemometric analysis has been frequently used in combination with FTIR spectroscopy for quantitative analysis [16]. In addition, the fatty acid compositions appear to be a useful technique for monitoring the adulteration practices of high price oils [12]. In this research, we developed an FTIR spectroscopic method combined with multivariate calibrations of PLS and PCR for quantitative analyses of NSO adulterated with GSO.

2. Experimental section

2.1. Materials

Nigella sativa L. seed oil (NSO) and other vegetable oils, including grape seed oil (GSO) were purchased from the local market in Selangor, Malaysia. These both oil samples were packaged in polyethylene terephthalate (PET) bottles and manufacturing date was identified as August 2010 and expiration date was at July 2014. The standard of fatty acid methyl esters (FAMEs) of 37 compounds (C4 to C24) was bought from Sigma Chemicals, St. Louis, MO, USA. All chemicals and reagents used were of analytical grade.

2.2. Preparation of oil samples

For FTIR spectroscopy analysis, the calibration and prediction samples composed of binary mixtures of NSO and GSO were prepared separately in the concentration ranges of 0.5–60.0% (v/v).

2.3. Fatty acid analysis

Fatty acid (FA) compositions of NSO and other vegetable oils were determined using a gas chromatograph (Shimadzu GC-2010, Shimadzu Corp., Tokyo, Japan), equipped with flame ionization detector.

The oven temperature was programmed as follows: the initial temperature was 100°C (hold for 1 min), then ramped into 180°C (8°C/min), increased from 180 to 240°C (10°C/min) and finally held at 240°C for 5 min. The temperatures of detector and injector were maintained at 240°C during the analysis. The flow rate of carrier gas (helium) was 6.8 ml/min. Before being analyzed, the samples of oils were treated with sodium methoxyde to form FAMEs forms according to method described by Chin et al. [9]. The column, oven and other conditions used during FA analysis are similar with those reported in Rohman et al. [23]. The qualitative analysis of FAMEs in samples was carried out by comparing retention times of the peaks with those of FAMEs standards. Quantification of FAs was performed using the technique of internal normalization and expressed as percentage based on peak areas.

2.4. Measurement of FTIR spectra

FTIR spectra of oil samples (either pure or admixtures) were measured using a Nicolet 6700 FTIR spectrometer (Thermo Nicolet Corp., Madison, WI, USA) equipped with deuterated triglycine sulphate (DTGS) detector and KBr/Germanium as beam splitter. The instrument was connected to the OMNIC software. The sampling compartment was a Smart Attenuated Total Reflectance accessory with dimensions of 10 × 60 mm, producing 12 internal reflections with a penetration depth (infrared beam) of 2.0 μm, composed of zinc selenide (ZnSe) crystal having refractive index of 2.4 at 1000 cm^{-1}. FTIR spectra were collected at mid-infrared region (4000–650 cm^{-1}), using 32 scans at a resolution of 4 cm^{-1}. These spectra were subtracted against the background of air spectrum. After every scan, a background of new reference air spectrum was taken. These spectra were recorded as absorbance values at each data point in triplicate.

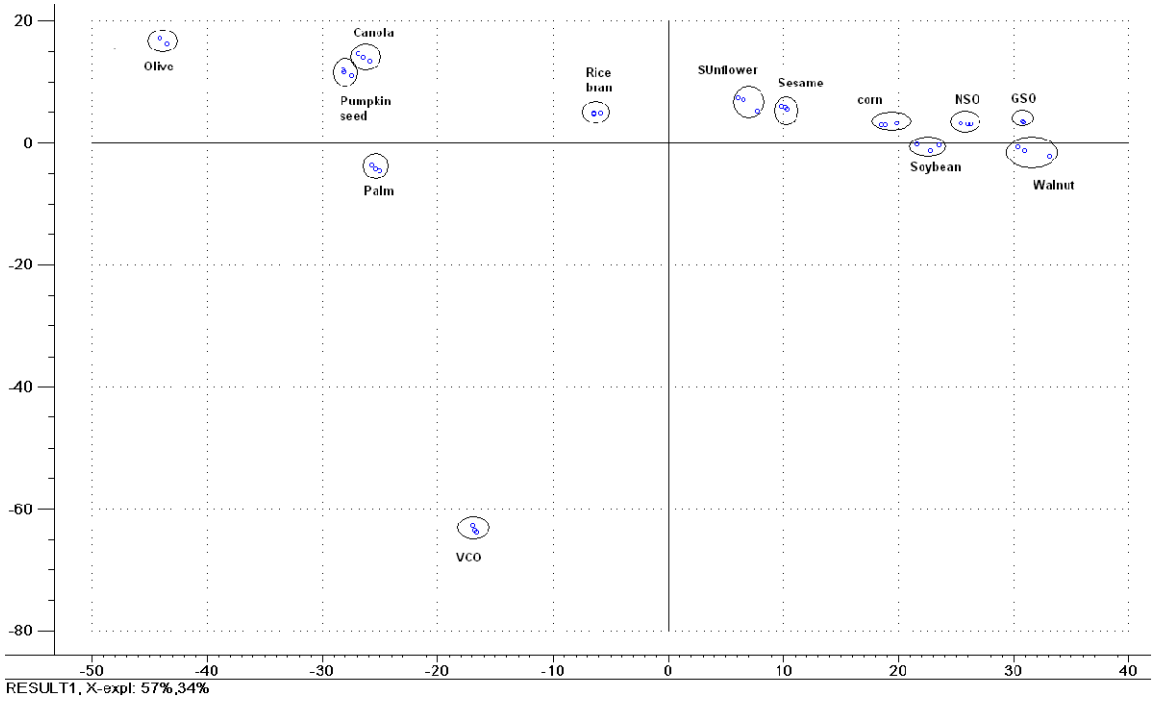
2.5. Statistical analysis

PCA of NSO and other oils using fatty acid profiles as variable matrix was performed with the aid of The Unscrambler version 9.7 software from Camo, USA. The multivariate calibration for FTIR spectral data was done by TQ Analyst™ software included in FTIR spectrophotometer.

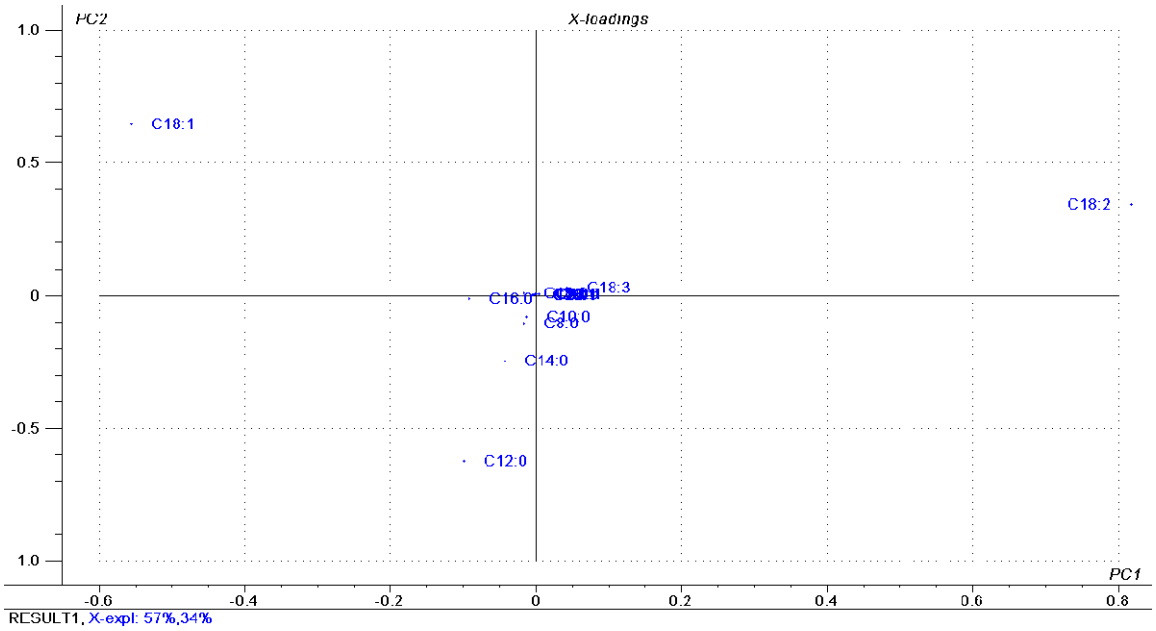
3. Results and discussion

In order to select the oil which is potential adulterant of *Nigella sativa* L. seed oil, PCA was used based on FA profiles as variables. PCA is one of the supervised pattern recognition techniques and is frequently used as the first step of the descriptive data analysis to distinguish the group of plant oils [13]. Figure 1(a) shows the score plot of PCA for classification of NSO and other common plant oils. According to PCA, about 91% variance can be explained using first (PC1) and second (PC2) principal components, in which PC1 describes 57% variance, meanwhile PC2 covers 34% variance.

NSO is located at the positive side, both in terms of PC1 and PC2, of PCA score plot. The nearest distance of plant oils to NSO is grape seed oil (GSO), meaning that GSO has similar fatty acid profiles with NSO; hence, GSO is potential oil adulterant in NSO. According to loading plot as shown in Fig. 1(b), it can be seen that linoleic acid (C18:2) has more influence on PCA score plot of NSO and GSO. The loading plots can be understood as the variable giving weight contribution to the principle component analysis (or to the objects will be classified). The FA composition of NSO and GSO are shown in Table 1. In addition, the FA profiles of other plant oils were reported in our previous paper [6]. The FA profiles of GSO were in agreement with those stated in [10].



(a)



(b)

Fig. 1. (a) PCA score plot for classification of NSO and other oils. SeO – sesame oil; CO – corn oil; RBO – rice bran oil; GSO – grape seed oil; PO – palm oil; PKO – pumpkin oil; EVOO – extra virgin olive oil; SO – soybean oil; SFO – sunflower oil; CaO – canola oil; WO – walnut oil; VCO – virgin coconut oil. (b) PCA score plot for classification of NSO and other oils in fatty acid profiles. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2011-0509.)

Table 1
The fatty acid compositions of Nigella seed oil (NSO) and grape seed oil (GSO)

FA†	GSO	NSO
C14:0	0.121 ± 0.007	0.048 ± 0.001
C16:0	9.219 ± 0.062	7.711 ± 0.038
C18:0	2.741 ± 0.208	3.513 ± 0.013
C18:1	36.498 ± 0.203	21.767 ± 0.011
C18:2	47.805 ± 0.123	65.310 ± 0.164
C18:3	0.307 ± 0.001	0.805 ± 0.008
C20:0	0.332 ± 0.002	0.212 ± 0.001
C20:1	0.580 ± 0.003	0.166 ± 0.001

Notes: FA – fatty acid; †each value in the table represents the means of triplicate analysis.

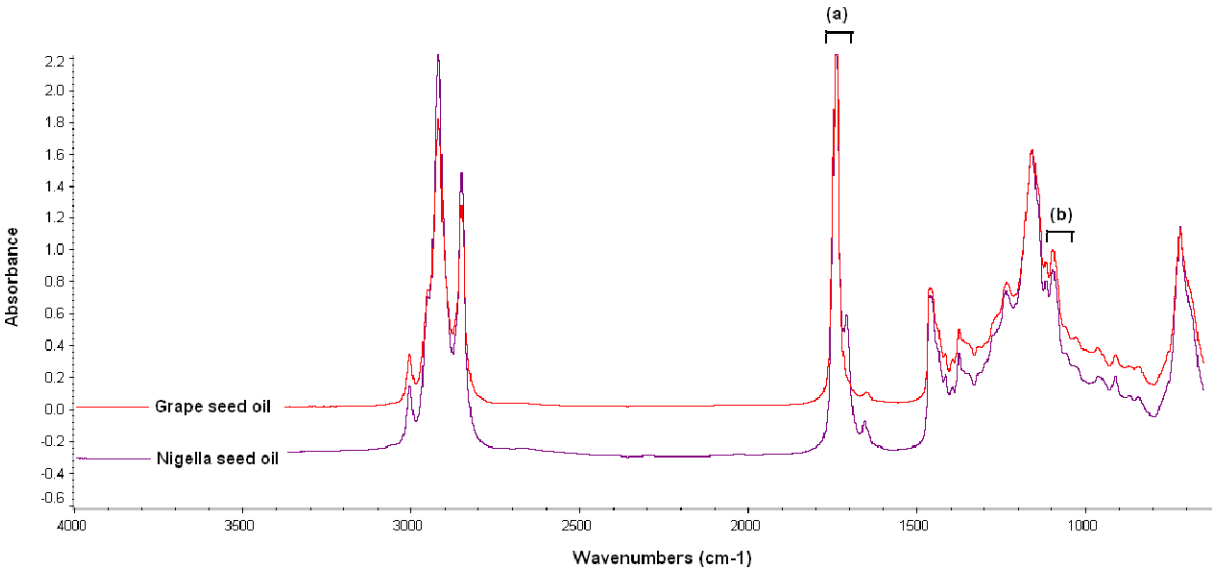


Fig. 2. FTIR spectra of *Nigella sativa* L. seed oil and grape seed oil at mid-infrared region range of 4000–650 cm^{-1}. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2011-0509.)

3.1. Analysis using FTIR spectroscopy

Figure 2 exhibits FTIR spectra of NSO and GSO at mid-infrared region range of 4000–650 cm^{-1}. FTIR spectra of both oils appear fairly similar. However, careful examination of FTIR spectra of both oils reveals some significant differences either in number of peaks at region 1750–1700 cm^{-1} (assigned with a) or in the peak intensities at frequencies of especially at 1117 and 1098 cm^{-1}, marked with b in Fig. 2. In the ranges of 1750–1700 cm^{-1}, NSO has two sharp peaks in 1744 and 1710 cm^{-1}; meanwhile GSO has one peak at 1744 cm^{-1}. These peaks were attributed to carbonyl (C=O) stretching vibration [26]. The functional groups responsible for absorption of IR by the common edible fats and oils have been reported in our previous papers. These frequency regions were further optimized in order to quantify the level of GSO in NSO with the aid of multivariate calibration [4].

The first step in quantitative analysis using multivariate calibration involves the selection of frequency region. The frequency region selected should include information providing the concentration variation in oil of interest and other oils in the mixtures, while excluding of spectral regions dominated by noise that might be built-in into the model [3]. This is carried out by computing the coefficient of correlation (R^2) determining the peak absorbancies at selected frequency regions to the concentration of oils. FTIR spectral (frequency) regions contributed to the high correlation between the actual and FTIR predicted values (high R^2) were selected; meanwhile regions that reveal low R^2 should be ignored [4].

Taking into account the high R^2 value (for the relationship between actual and calculated value using FTIR spectra) and the lower error in calibration (expressed as root mean square error of calibration or RMSEC), the combined wavelength number of 1114–1074, 1734–1382 and 3005–3030 cm^{-1} were chosen for analysis of GSO in NSO. Partial least square (PLS) model was exploited to facilitate the quantitative analysis of GSO in NSO. The scatter plot describing this relationship together with the residual plot (difference between actual and calculated values) were shown in Fig. 3(a) and (b), respectively.

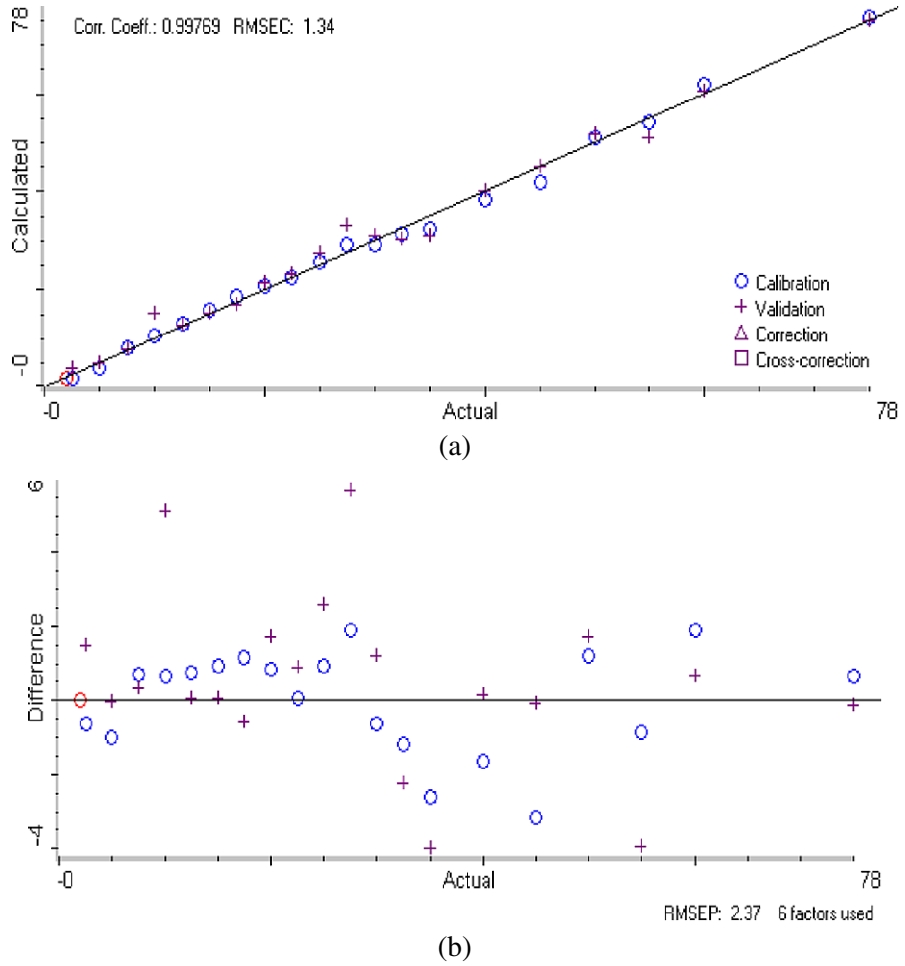


Fig. 3. (a) The scatter plot describing the relationship between actual (x-axis) and FTIR predicted (y-axis) values. (b) The residual (difference between actual and predicted values) of GSO. (Colors are visible in the online version of the article; http://dx.doi.org/10.3233/SPE-2011-0509.)

In order to check of the performance of the PLS calibration model, we used two approaches. Firstly, a cross validation using "leave-one-out technique" was explored. In this technique, one GSO sample in PLS calibration samples was removed. The remaining calibration samples were used to predict the level of removed samples and the error between actual and calculated values of the removed sample is calculated. This process is repeated until all calibration samples were removed one by one. The total error, expressed as root mean square error of cross validation obtained is 2.34% (v/v) with R^2 of 0.981. Secondly, we use a set of independent samples called as prediction/validation samples. PLS calibration model was further used to evaluate the level of the prediction samples. The error, expressed as root mean square error of prediction (RMSEP) obtained is 2.37% (v/v). The difference between actual and FTIR calculated values of GSO in prediction samples was also included in Fig. 3(b).

PLS calibration model is also called "factor analysis", because this technique relies on the linear combination of the FTIR spectral variables called as factors or "principal components or PCs" rather than original spectra [20]. The software TQ Analyst was designed to select the number of PCs appropriate for quantitative analysis automatically. The number of PCs suggested is 6 because this number provides the minimum level of predicted residual error sum of square (PRESS) during cross validation for quantifying GSO in NSO. Based on the results in terms of high value of R^2 in calibration and prediction samples as well as the low value of errors, it deems that FTIR spectra coupled with PLS is powerful enough for the monitoring the adulteration practice of GSO in NSO.

4. Conclusion

It can be concluded that the combination of two analytical techniques of FTIR spectroscopy and gas chromatography can be an effective mean for detecting the adulteration practice of NSO with GSO. Using FTIR spectroscopy, the levels of GSO can be quantified using the combined frequency regions of 1114–1074, 1734–1382 and 3005–3030 cm^{-1} with the aid of partial least square.

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