

Research Article

SB-ATR FTIR Spectroscopic Monitoring of Free Fatty Acids in Commercially Available *Nigella sativa* (Kalonji) Oil

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Free fatty acids (FFA) in *Nigella sativa* (*N. sativa*) commercial and seed oil were determined using single-bounce attenuated total reflectance (SB-ATR) Fourier transform infrared (FTIR) spectroscopy. Gravimetric mixing was done by adding 0.1–40% oleic acids in neutralized *N. sativa* oil containing 0.1% FFA. FTIR spectroscopy technique and partial least square (PLS) calibration were used to detect the absorption region of carbonyl (C=O) which is in the range of 1690–1727 cm^{-1} . The results of PLS calibration model and root mean square error of calibration (RMSEC) are 0.999 and 0.449, respectively. Comparing the FFA obtained in *N. sativa* oil by using FTIR with the FFA obtained using AOCS titrimetric method shows a positive correlation and confirms that the described method is a useful procedure.

1. Introduction

Herbal products are increasingly used as alternatives to orthodox drugs or medicine. *Nigella sativa* plant belongs to the Ranunculaceae family and it is commonly found in South, Southwest Asia, North Africa, and Southern Europe where it has been used traditionally as medicinal plant and spice since ancient time [1]. In Pakistan and India, *N. sativa* is cultivated as an annual herb and is commonly called kalonji [2]; other names are black cumin or black seed or Roman coriander or nutmeg flower or fennel flower [3]. It has been commonly used in traditional medicine as natural remedy for various diseases for over 2000 years in the Middle Eastern folk medicines [4]. *N. sativa* is used as seasoning and flavouring supplement for food, bread, pickles, and bakery products [5].

N. sativa oil or extract has protective and curative actions. Extracts of *N. sativa* have been used for the treatment of hypertension, asthma, inflammation, diabetes, eczema, bronchitis, fever, headache, dizziness, cough, and influenza [6, 7]. It has been reported in the literature that seed of *N. sativa*

found to be diuretic, carminative, stimulant, galactagogue and emmenagogue, and antipyretic [8].

Oil is a complex mixture that contains fatty acids, vitamins, pigments, volatile, and antioxidative components. The hydrolysis of oil results in the formation of FFA and glycerol residues. Among the oil quality parameters, FFA is the major cause of flavor deterioration and shelf life of the oil [9]. Therefore, it is a crucial factor associated with the quality and economic value of edible oils, especially for unrefined high value oils such as olive and *N. sativa* oil.

Generally, FFA content is determined by titration method. According to the procedure, oil is dissolved in diethyl ether or neutralized ethanol and titrated with a strong base in the presence of phenolphthalein as an indicator [10, 11]. Although the method is sensitive, but involves toxic chemicals and laborious; furthermore, there are some chances of errors in determination of the end point of dark colored oils [12]. To avoid these common problems, number of instrumental methods such as colorimetric [13], voltammetric [14], flow injection [15], gas and liquid

chromatography [16, 17], and infrared spectroscopic [18] methods have been reported for the determination of FFA in vegetable oils.

Compared to other instrumental techniques, FTIR was found to be rapid, nondestructive, and easy technique. Moreover, less sample preparation is required, thus reducing the cost of chemicals and solvents. Due to these applications FTIR is also considered as “green analytical tool” [19]. FTIR has been successfully applied for the determination of various parameters of oils and fats such as per oxide [20], *trans* fatty acids [21], adulteration [22], fatty acid composition [20, 23], fatty acid ratio [24, 25], classification & authentication [26], conjugated diene & triene [27], and tocopherol [28].

To the best of our knowledge, no literature report has been published on the evaluation of *N. sativa* oil by FTIR spectroscopy. The objective of this research was to evaluate the FFA content in *N. sativa* seed oil and commercially available oils in the market by using SB-ATR FTIR spectroscopy.

2. Materials and Methods

2.1. Reagents and Samples. All reagents used were of analytical grade. Oleic acid (99%) and sodium hydroxide were purchased from Fluka Chemie GmbH (Buchs, Switzerland). *n*-hexane was obtained from Fisher Scientific UK Ltd. and ethanol was purchased from Merck (Darmstadt, Germany).

2.2. Sample Preparation. *N. sativa* seed and *N. sativa* commercial oil samples were purchased from supermarkets of Hyderabad, Pakistan. About 20 g of ground *N. sativa* seed soaked in 100 mL of *n*-hexane for 6 hours at 70°C, oil was extracted using Soxhlet extraction method. The fixed oil was pooled and concentrated in a rotary evaporator (Buchi Rotavapor-RE 111), then placed in a vacuum oven at 105°C for 15 min, cooled in a desiccator and refrigerated until further analysis.

2.3. FFA Contents. The FFA contents of the *N. sativa* seed oil and commercial oils were determined by titrimetric method (AOCS Official Method Ca-5a-40, 1989) [10].

2.4. Preparation of FTIR Calibration Standards. A set of ten calibration standards covering an FFA range of 0.1 to 40% were prepared by gravimetric addition of oleic acid to neutralized *N. sativa* oil containing 0.1% FFA as determined by the standard AOCS titrimetric method.

2.5. Instrumentation. Infrared spectra were acquired using a Thermo Nicolet 5700 FTIR spectrometer (Thermo Nicolet Analytical Instruments, Madison, WI) equipped with a pyroelectric deuterated triglycine sulfate (DTGS) detector. An SB-ATR accessory with a removable ZnSe crystal was mounted in the sample compartment. The FT-IR controlled by OMNIC software (version 7.2) and dataset was collected between 4000 and 650 cm^{-1} by coaddition of 32 scans at a resolution of 4 cm^{-1} . The fresh background spectrum recorded from the bare ATR crystal was subtracted from each standard or sample spectrum. Prior to collection of each

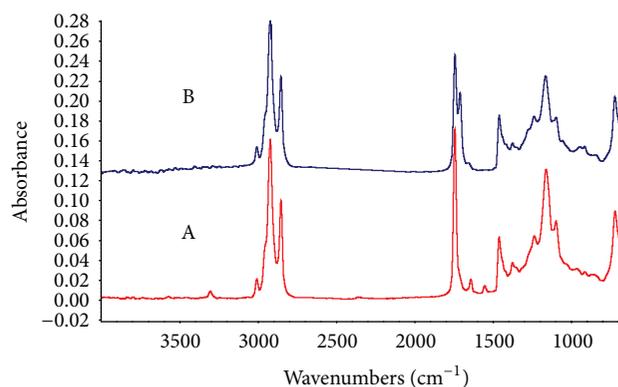


FIGURE 1: FTIR spectra of commercial *N. sativa* oil (A) and freshly extracted oil from *N. Sativa* seed (B).

background spectrum, the ATR crystal was carefully cleaned with propanol to remove any lipo- or hydrophilic residues of the previous sample, and residual solvent was evaporated using a stream of nitrogen gas.

2.6. Partial Least Squares Chemometric Analysis. Chemometric analysis was carried out using the Turbo Quant (TQ) analyst 7.2 software package from Nicolet (Madison, WI, USA). The spectra of FFA calibration in combination with reference FFA values were used by the software to develop PLS calibration in the range from 1727 to 1690 cm^{-1} . To assess the capability of the model to fit the calibration data and to calculate the deviation of the model, root mean square error of calibration (RMSEC), root mean square error of cross-validation (RMSECV), and root mean square error of prediction (RMSEP) were used as described earlier [27].

2.7. Accuracy and Reproducibility. The accuracy of the SB-ATR FTIR predictions was assessed in terms of mean difference (MDa) and standard deviation of the differences of accuracy (SDDa) as suggested by AOAC International [29] taking the titrimetric data as the true reference values. The reproducibility of the SB-ATR FTIR method was compared with that of the AOCS titrimetric method in terms of the mean difference (MDr) and standard deviation of the differences of reproducibility (SDDr) between triplicate analyses of each of the samples.

3. Results and Discussion

Figures 1(A) and 1(B) illustrate the mid-infrared spectra of *N. sativa* seed oil and commercial oil in the region 4000 to 650 cm^{-1} . The C–H stretching vibration of the *cis*-double bond (=CH) was observed at 3006 cm^{-1} [30]. The CH_3 and CH_2 asymmetric stretching vibrations were found in the region of 2900 and 2800 cm^{-1} , respectively [31]. The band at 1648 cm^{-1} is due to the absorption of C=C *cis* stretching. The CH absorption of bending vibrations CH_2 and CH_3 bands can be clearly seen in the region of 1465 and 1377 cm^{-1} , respectively, while the band at 723 cm^{-1} assigned to a CH_2

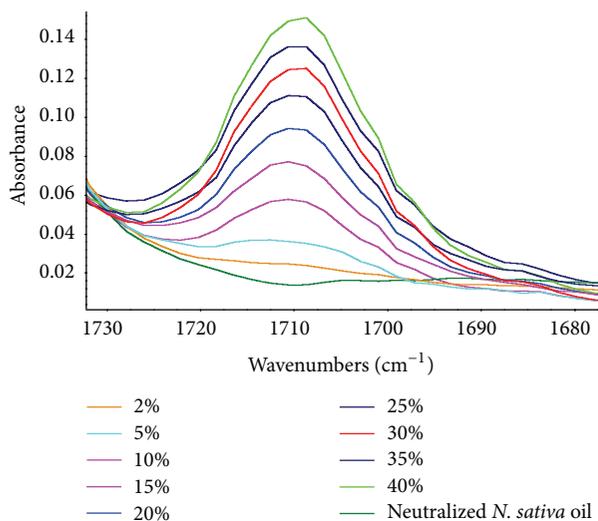


FIGURE 2: Group spectra of calibration standards spiked with oleic acid.

rocking vibration [31]. The spectra showed characteristic absorption bands associated with the common edible oil and both spectra found to be similar. On the other hand, careful examination of spectra reveals some significant variations in the region of 1750–1690 cm^{-1} . The bands at 1748 cm^{-1} and 1711 cm^{-1} are due to the triglyceride ester carbonyl absorption and C=O absorption of FFA, correspondingly [32]. The band at 1711 cm^{-1} is absent in Figure 1(A) (extracted seed oil), while in Figure 1(B) (commercial oil) it is very prominent and represent higher level of FFA.

PLS model was developed to calculate the level of FFA in freshly extracted *N. sativa* oil and commercially available *N. sativa* oil samples. Figure 2 represents the group spectra of calibration standards expended in the range of 1727–1690 cm^{-1} which shows absorption of a FFA band at 1711 cm^{-1} . Calibration standards were prepared in the range of 0.1–40%. For the PLS model out of ten calibration standards, seven of them were used as a calibration points and three used as a validation points (randomly selected by the software). Figure 3 shows the correlation coefficient of the fraction of the difference in the Y variable (calculated) FFA values predicted by FTIR that is accounted by the X variable (actual) calibration standards. The residual values of FFA concentration was determined from PLS regression.

Table 1 summarized other parameters determined from PLS calibration, which include the number of factors, correlation of coefficient (R^2), RMSEC, RMSECV, and the RMSEP in PLS calibration model. The number of factors used in the model was automatically selected by the TQ software, and the selection of factor was used to attain the lowest possible predicted residual error of sum of squares (PRESS) value.

Figure 4 shows the rejection of the outlier standard from calibration points. The rejection criterion was based on the maximum distance from the mean value which was 5. It was observed that none of the prepared calibration standard crossed the maximum distance value. All calibration points

TABLE 1: Prediction capabilities of PLS—FTIR measurement model for the determination of FFA.

Spectral region	1727–1690 cm^{-1}
Factors	4
Validation standards	3
RMSEC	0.449
RMSECV	2.265
RMSEP	0.324
R^2	0.9993

R^2 is correlation coefficient of actual and calculated values of free fatty acids concentration in the calibration set; RMSEC: root mean square error of calibration; RMSECV: root mean square error of cross-validation; RMSEP: root mean square error of prediction.

TABLE 2: FFA content (Mean \pm SD) of commercial and *N. sativa* seed oil by Titrimetric and SB-ATR FTIR Methods.

<i>N. sativa</i> Oil	% FFA Titration	% FFA FTIR
Seed oil	3.75 \pm 0.09	3.70 \pm 0.070
Sample 1	20.35 \pm 0.26	20.47 \pm 0.044
Sample 2	16.15 \pm 0.32	16.10 \pm 0.089
Sample 3	14.22 \pm 0.35	14.16 \pm 0.037
Sample 4	16.16 \pm 0.16	16.06 \pm 0.050
Sample 5	9.68 \pm 0.19	9.79 \pm 0.030
Sample 6	26.81 \pm 0.40	26.77 \pm 0.085
	MDr = 0.39	MDr = 0.09
	SDDr = 0.07	SDDr = 0.03

were found below 1.5, which revealed the correctness of the developed PLS model.

Table 2 presents a comparison of AOCS titrimetric results with those carried out by the FTIR method. Both MDr and SDDr for reproducibility were considerably higher for the titrimetric procedure than the FTIR method, the FTIR triplicates tending to the same mean without any significant bias MDr 0.09 versus 0.39% FFA with less variability around the mean difference SDDr 0.03 versus 0.07. Figure 5 shows the linear correlation between the mean values of the triplicate analysis of the two methods FTIR and titrimetric, the MDa and SDDa of accuracy being 0.01% FFA and 0.09% FFA, respectively. If we look at level of the commercial *N. sativa* oil samples, it was noticed that the entire samples contained higher amount of FFA as compared to the freshly extracted *N. sativa* seed oil, which could not be recommended for the edible purposes.

4. Conclusion

The SB-ATR FTIR spectroscopic procedure has shown substantial advantages for the FFA monitoring in *N. sativa* oil over standard AOCS titrimetric method in terms of simplicity and speed of analysis. Furthermore, no solvents or any other chemical is required and only one drop of oil is sufficient for FFA determination by FTIR. Higher contents of FFA in commercial *N. sativa* oils were observed as compared to the extracted oil which is worrisome matter for the consumers.

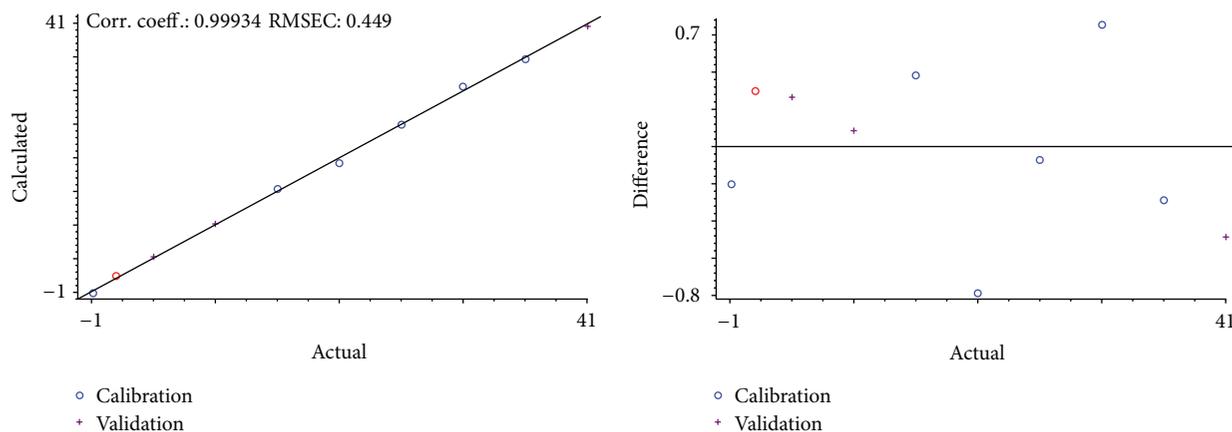


FIGURE 3: Relationship plot of actual FFA versus predicted FFA by FTIR with their differences.

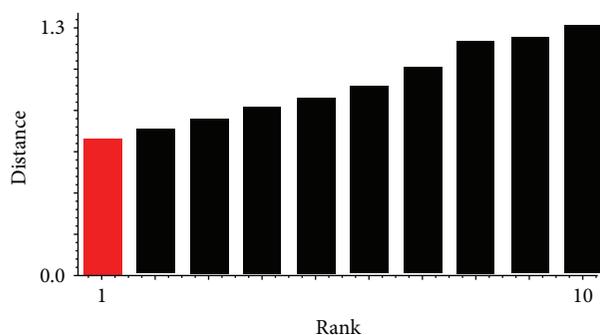


FIGURE 4: Representation of outlier calibration standards.

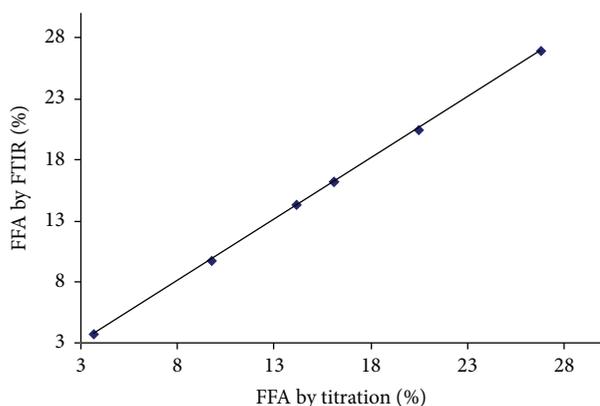


FIGURE 5: Correlation plot of mean AOCS titrimetric method versus SB-ATR FTIR predicted FFA.

Conflict of Interests

The authors declare that there are no conflicts of interests.

Acknowledgments

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