Quantification of Pure Refined Olive Oil Adulterant in Extra Virgin Olive Oil using Diamond Cell ATR-FTIR Spectroscopy

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Summay: The present study depicts spectroscopic method development to deliver a rapid, simple and reproducible quantification of pure refined olive oil (PROO) adulterant in extra virgin olive oil (EVOO) using partial least square (PLS) regression (statistical parameter). Single bounce attenuated total reflectance (SB-ATR) Fourier transform infrared (FTIR) was choice in the developed method. Blended standards of PROO and EVOO were obtained by their weight by weight percentage and the values were used to construct calibration curves for quantification. The optimum regression values (i.e. >0.99) were achieved using the combined frequencies of 3105-2761, 1838-1687, and 1482-440 cm^{-1} with regression coefficients (R^2) 0.99718 and achieved residual mean square error of calibration (RMSEC) 1.40% w/w. To determine the suitability of developed method principal component spectra (PCS) diagnostic was also used. The results of the present study prove that the developed methods reported in preceding studies can be good option for more rapid and accurate determination of PROO adulteration in EVOO.

Key words: Extra virgin olive oil, Adulteration, FTIR, Chemometrics.

Introduction

The International Olive Oil Council, Olive oil can be classified into various grades [1]. Of those, mainly are virgin olive oils (i.e. ordinary virgin olive oil extra virgin olive oil and virgin olive oil), olive–pomace oils (i.e. crude olive–pomace oil, refined olive–pomace oil and olive–pomace oil) and refined olive oils. The significant difference among the prices of oils has motivated adulteration of costly oils with cheaper oils. Though such mixing of cheaper oils in costly oils does not cause anything that may be associated with health problem, however the primary consumer is deprived of his rights which are violated by such deceiving practices [2]. Best example of the said practice is adulteration of extra virgin olive oil (EVOO) which is highly priced and mostly adulterated by mixing of low-grade olive oils, olive–pomace oil or refined olive oil as well as other cheaper vegetable oils such as hazelnut oil, sunflower oil, soybean oil and maize oil [3]. Thus the quantification of adulterants of EVOO has been desired in scenarios after highlighted above.

Detection of refined olive oil and pomace oil adulteration in EVOO often becomes difficult to accomplish, especially when oils with chemical similar compositions are added [4]. As a result it was conceived that new methods should be developed for the determination of adulteration of EVOO.

Different analytical methods have been employed to detect adulterants virgin in EVOO. Most of these are based on chromatography (gas chromatography, high performance liquid chromatography), spectroscopy (ultraviolet, near-infrared (NIR), mid-infrared, visible, Raman), isotopic analysis and electronic nose systems [5-8]. Relevant applications of the chemometric techniques and electronic nose have also been reviewed [3, 9-11]. It is suggested that using chemometric analysis in the NIR, adulteration of pure olive oil with soybean, sunflower, corn, walnut and hazelnut oils could conveniently be predicted [5]. Qualitative and quantitative determination of vegetable oils (canola, hazelnut, pomace and high linoleic/oleic sunflower) as adulterants in commercial samples of EVOO has been reported [12].

In the study of edible fats and oils FTIR spectroscopy has been used as a powerful analytical tool, especially for qualitative characterization of specific components in foods [13].

The use of diamond cell ATR-FTIR spectroscopy for the quantification of pure refined olive oil (PROO) adulterant in EVOO has been the approach in this study. The assessment of the capability of diamond cell ATR-FTIR coupled with Turbo Quant (TQ) Analyst chemometrics such as partial least square (PLS) and principal component spectra (PCS) to discriminate the EVOO mixed with PROO was the main objective of this study. This approach displays a facile and convenient means for monitoring EVOO quality. The advantages of this technique are ease of operation, high sample turnover and no sample pretreatment.

Results and Discussion

A unique spectral fingerprinting of the infrared spectrum of organic molecules provides detailed information about their molecular structure. However, this unique fingerprint becomes confusing when similar molecules containing structural features are mixed to each other, e.g. in fats and oils, complex mixture of triacylglycerols [14, 15].

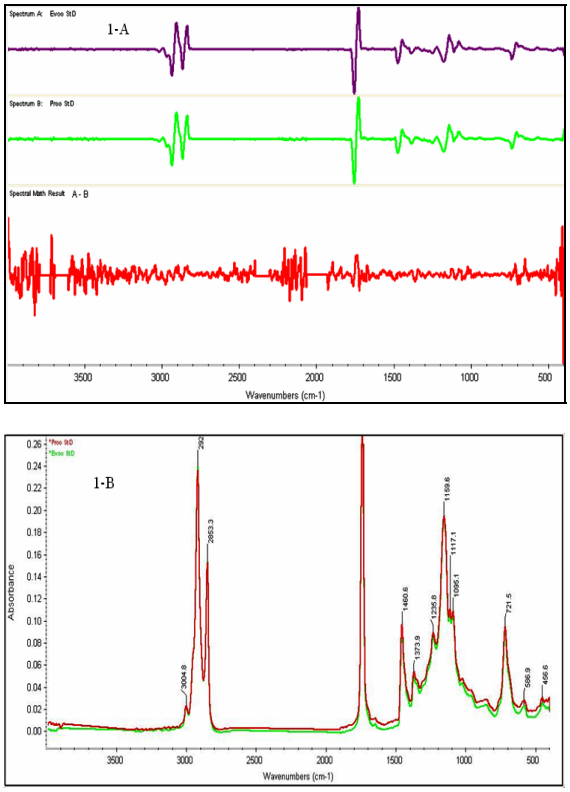

Fig. 1: (A and B) ATR-FTIR spectra (1st derivative difference) and normal spectrum of EVOO and PROO.

Fig. 1-A, is representative of first derivative spectrum of EVOO and PROO standards and resultant spectrum obtained by the subtraction of PROO from EVOO with a spectral math parameter, shows the clear dissimilarity between characteristic bands. The band at 3005 cm^{-1} shows (–C–H) stretching vibration of cis- double bond of unsaturatured fatty acids Fig. 1-B, while symmetric and asymmetric vibration of aliphatic $–CH_2$ hydrocarbon chains represents the characteristics bands at 2922 and 2853 cm^{-1} [15]. Major peak at 1744 cm^{-1} arises from C=O stretching vibrations, the peak is associated with the triglyceride ester-linkage (COOR) band and the C=O absorption of free fatty acid present in the olive oil. The band at 1461 cm^{-1} is attributed to asymmetric stretching in methyl and methylene groups, while the peak at 1160 cm^{-1} is associated with the stretching of the C–O bonds of aliphatic esters [16-18]. The finger print region plays a very important role in the identification of the variation among the bands.

Fig. 2-A and 2-B represent the ATR-FTIR spectra of normal and first derivative of 12 blended oils respectively, which clearly show the variation in the absorption bands could be related to compositional differences among oil groups. The spectra did not show an obvious difference from visual inspection according to the varietal regions. However, PLS algorithm can easily predict these minor variations in the spectrum.

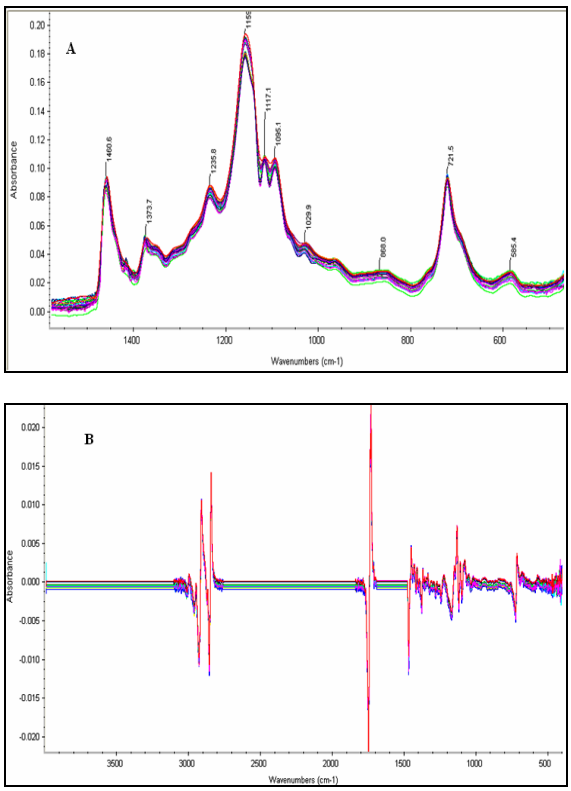

Fig. 2: (A and B) Normal and first derivative FTIR spectra of all different ratios (EVOO vs. PROO) oils in the selected spectral region (1500–450cm^{-1})

Table-1 lists the parameters used to statistically assess the results of calibration model to determine PROO percentage in EVOO by using normal mid-ATR-FTIR spectra. The calibration model performed properly yielding good correlation coefficients and low residual mean standard error of calibration (RMSEC) values. The assessment of the

errors was carried out by calculating RMSEC in the calibration model after comparing the actual concentration with those computed for each component.

Table-1: Abilities of calibration and prediction model for PROO adulterant in EVOO by ATR-FTIR.

Region type	Spectral range (cm^{-1})	Base line type	Factors	R^2	RMSEC
First Derivative in range	3105-2761 1838-1687 1482-440	One point Average in range One point Fixed location Two points Fixed Location	4	0.99718	1.40
Spectrum in range	3105-2761 1838-1687 1482-440	One point Average in range One point Fixed location Two points Fixed location	4	0.99178	2.38
Spectrum in range	3141- 2819 1484- 493	Two points Average in range Two points Average in range	4	0.95558	5.09
Spectrum in range	3105- 2761 1838-1687	One point Average in range One point Fixed location	2	0.8816	6.78
Spectrum in range	1482-440	Two points Fixed location	3	0.99073	2.03

R^2 is regression coefficient of actual and calculated values for blended oil calibration set. RMSEC, root mean square error of calibration.

Full region (4000-450 cm^{-1}) was selected prior to three different selective regions (3105-2761), (1838-1687), (1482-440) of the mid IR spectrum which were taken to construct PLS calibration, individually each region does not provided satisfactory results in term of determination coefficient (R^2) and RMSEC. However, combined frequencies of these regions (3105-2761, 1838-1687 and 1482-440), and the baseline types for these spectral ranges were optimized as one point (average in range), one point (fixed location) and two points (fixed location) respectively, with first derivative in each region also selected. The determination coefficient (R^2), RMSEC for calibration set were 0.99718; 1.40 respectively, therefore the values of R^2, RMSEC of developed calibration proves the simplicity of method. Correspondingly for calibration model these values were within the acceptable range.

Table-2 shows the mean percentage adulteration of PROO in EVOO of eight commercial extra virgin olive samples determined by ATR-FTIR spectroscopy. Amongst the analyzed EVOO samples, the highest amount was determined in CS-4, (26.19%), while lowest in the CS-3, 5.44%, whereas sample CS-6 to CS-8 were lower from the detection range of the calibrated method.

Table-2: ATR-FTIR determination of PROO adulteration in EVOO of eight commercial samples

Samples	Percentage by FTIR mean ± St. Dev.
CS-1	11.23 ±0.25
CS-2	16.12 ±0.35
CS-3	5.44 ±0.16
CS-4	26.19 ±0.42
CS-5	23.26 ±0.64
CS-6	2.3/ND
CS-7	1.5/ND
CS-8	3.2/ND

CS, commercial samples, ND, not determined

The spectral information can be used as a measurable property which could make possibility of establishing a calibration, thus, advantage of using advanced chemometric techniques such as PLS is there [14]. The information provided by the calibration results diagnostic can help in identifying standards that may be outliers. A typical percentage difference plot will show data points distributed randomly above and below the zero line within a narrow concentration range.

Fig. 3 presents the calibration plot and percentage difference plot between actual and predicted values. In developing the PLS model, the percent values for standard oils obtained from pre-constituted mixtures of EVOO with PROO (w/w) were put along with the spectra into the Turbo Quant (TQ) Analyst program. At the time of the optimization process, the combined frequency regions of 3105-2761, 1838-1687 and 1482-440 cm^{-1} were selected. The developed calibration model offers highest values of R^2 and lowest value of RMSEC.

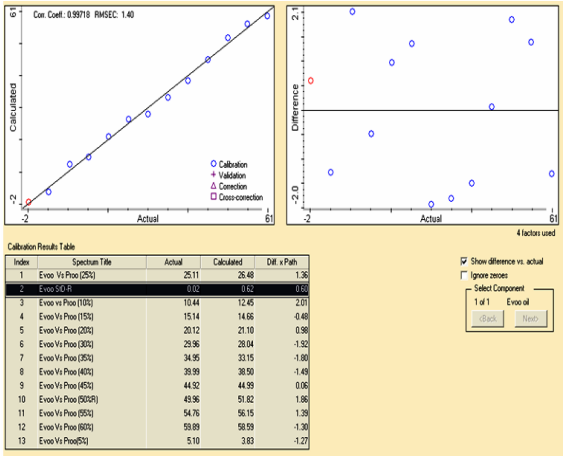

Fig. 3: FTIR-PLS calibration plot and percent difference between actual and predicted value of blended oil.

Principal component spectra (PCS) diagnostic has also been employed. Ten PCS of blending samples/standards were obtained using the advanced diagnostic option in Turbo Quant Analyst software (Fig. 4). These noisy or featureless PCS indicate that the corresponding (and any subsequent) principal component contributes little useful information to the calibration model. The PCS show how the spectral information in a calibration set is represented by the principal components. PCS is the orthogonal spectrum that represents the amount of variability described by a principal component measured across the entire spectral range of the standards. The data obtained from these spectra were put into the Microsoft excel software to obtain a calibration plot between percentage variance against cumulative percentage variance with R^2 value at 0.973 which further confirmed the reliability and accuracy of data (Fig. 5).

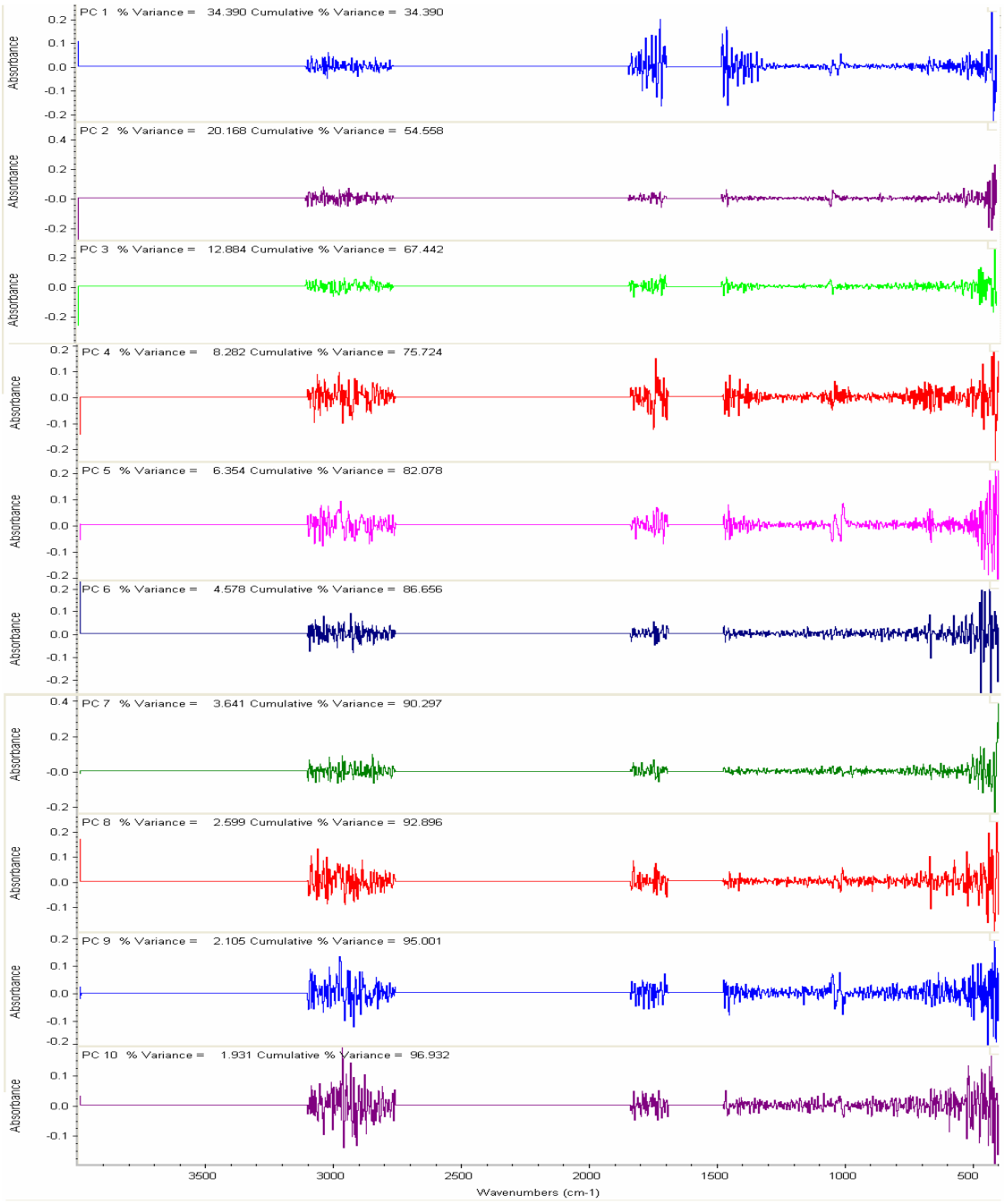

Fig. 4: Principal component spectral diagnostics of blended standards.

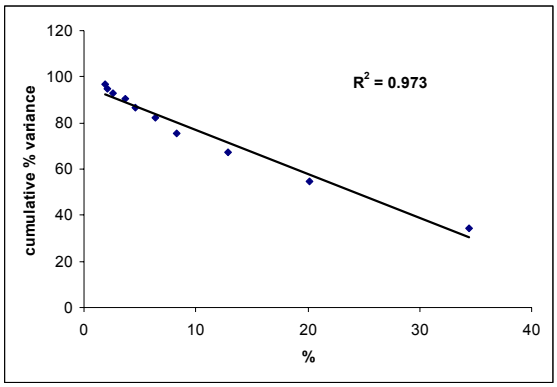

Fig. 5: Principal component spectral diagnostic plot between percentage variance and cumulative percentage variance

Experimental

Samples and Reagents

EVOO and PROO were bought from the local industry in Karachi, Pakistan. The oil samples were stored in glass bottles in dark before being used for analysis. The dates of manufacturing and expiry of samples were also mentioned. All chemicals (e.g. reagents and solvents) to be used in the study were purchased from E. Merck (Darmstadt, Germany).

Blending of Oils for ATR-FTIR Analysis

PROO was added into EVOO in the range of 5–60% w/w, at interval of 5 units (Table-3). The blended samples were kept in controlled room temperature 25 ^{0}C during authentication studies.

Table-3: Pre-constructed blending (w/w) of PROO in EVOO.

Samples	% Blending	EVOO (A)	PROO (B)	x=A+B	(A/x)×100	(B/x)×100
1	5%	90.50	05.51	100.01	94.90	5.10
2	10%	90.02	10.05	100.07	89.56	10.44
3	15%	80.51	10.52	100.03	84.87	15.13
4	20%	80.03	20.02	100.05	79.88	20.12
5	25%	70.52	20.52	100.04	74.89	25.11
6	30%	70.05	30.01	100.06	70.04	29.96
7	35%	60.54	30.51	100.05	65.05	34.95
8	40%	60.02	40.01	100.03	60.01	39.99
9	45%	50.52	40.50	100.03	55.08	44.92
10	50%	50.02	50.01	100.03	50.04	49.96
11	55%	40.55	50.51	100.07	45.24	54.76
12	60%	40.03	60.02	100.04	40.11	59.89

FTIR Spectral Measurements

Infrared spectra of the blended samples were recorded on a Thermo Nicolet Avatar 320 FTIR spectrometer. It was equipped with a Diamond Cell Smart Accessory (ID: 060-5013) which was removable. The detector was deuterated triglycine sulfate (DTGS) and KBr optics. For data acquisition and instrument control, OMNIC software version 7.0 (Thermo Nicolet Analytical Instruments, Madison, WI) from Thermo was employed. All spectra were collected by co-addition of 32 scans at a resolution of 4 cm^{-1} in the range of 4000–400cm^{-1} at 1.93 data spacing. The spectrum of each standard or sample was ratioed against a fresh background spectrum recorded from the uncovered removable diamond crystal. All analyses were carried out at room temperature, and three spectra were recorded for each sample. ATR crystal was carefully cleaned with a cellulose tissue soaked in n-hexane and then rinsed with acetone to remove any lipo- or hydrophilic residues of previous sample. The main benefits of using a diamond cell ATR smart accessory is its simplicity in handling, It only requires a sample to be placed on the crystal and the spectrum is taken against the fresh background of the clean crystal.

FTIR Calibrations

The FTIR calibration set consisted of 12 blended oil samples/standards spectra, these spectra, along with their respective reference percentage (w/w) were input into the TQ Analyst program to develop PLS calibrations. The performance of calibration was assessed by linear regression, and evaluated by running the samples of known percentages of blends. Therefore, in the calibration step, partial least square (PLS) regression and PCS diagnostics were applied for developing the calibration models. To assess statistical correctness of PLS models, correlation coefficient and deviation of models between predicted and reference values, such as RMSEC (Fig. 3).

Statistical Analysis

Each sample was divided in two and run in triplicate by OMNIC software version 7.0 package from Nicolet (Madison, WI, USA). The results were put in the Turbo Quant (TQ) to develop PLS calibration and PCS diagnostic and reported as mean ± SD (n=2×3).

Conclusion

The present approach indicated that the diamond cell ATR-FTIR spectroscopy can be a suitable tool for the determination of PROO adulterant in EVOO oil samples. No costly standard, reagent and chemicals are required in applying the developed method for analysis of samples. Thus, it is

concluded that the method is simple, sensitive and reproducible after the stabilization of the instrument under optimized environmental conditions, especially temperature and humidity. For the determination of adulteration in oils the proposed method could be easily applied.

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