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Simultaneous quantitative analysis of two functional food oils, extra virgin olive oil and virgin coconut oil using FTIR spectroscopy and multivariate calibration

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Abstract: Two functional food oils, namely extra virgin olive oil (EVOO) and virgin coconut oil (VCO) have been analyzed simultaneously using Fourier transform infrared (FTIR) spectroscopy. The performance of multivariate calibration of principle component regression (PCR) and partial least square regression (PLSR) was evaluated in order to give the best prediction model for such determination. FTIR spectra were treated with several treatments including mean centering (MC), derivatization, and standard normal variate (SNV) at the combined frequency regions of 3050 – 3000, 1660 – 1650, and 1200 – 900 cm^{-1}. Based on its capability to give the highest values of coefficient of correlation (R) for the relationship between actual value of EVOO/VCO and FTIR predicted value together with the lowest values of root mean square error of calibration (RMSEC), PLSR with mean centered-first derivative spectra was chosen for simultaneous determination of EVOO and VCO. It can be concluded that FTIR spectroscopy combined with multivariate calibration of PLSR was successfully applied to simultaneously quantify EVOO and VCO with acceptable parameters.

Keywords: Extra virgin olive oil, virgin coconut oil, partial least square regression, principle component regression, FTIR spectroscopy

Introduction

Today, olive oil and virgin coconut oil (VCO) are among valuable oils due to its potential effect toward the human health, therefore both oils can be considered as functional food oils. Marina *et al*. (2009) have reviewed some aspects related to VCO; meanwhile the beneficial effects of olive oil were described by García-González *et al*. (2008).

Olive oil is a fatty juice and is straightforwardly consumable after the proper processing of olives and has gained the popularity in recent years (Arvanitoyannis and Vlachos, 2007). It is consumed not only by the people in the Mediterranean countries but also by community worldwide because of its unique flavor, high content of oleic acid (monounsaturated fatty acid or MUFA) which is beneficial to health, and the presence of minor components such as antioxidants which are important to the biological activities (Boskou, 2009). Due to this reason, olive oil commands a high price on the market (Li-Chan, 1994). Edible olive oils are graded into six categories, namely (i) extra virgin olive oil (EVOO) with acidity up to 0.8%, calculated as oleic acid; (ii) virgin olive oil (acidity about 2.0%); (iii) refined olive oil with free acidity of 0.3%; (iv) common olive oil (a mixture of refined olive oil and virgin olive oil); (v) refined residue oil, and (vi) residue olive oil (Boskou, 2009). EVOO is considered as the best olive oils because EVOO is obtained from the mechanical extraction and is not treated with the artificial processing (Piravi-Vanak *et al*., 2010).

In addition, VCO can be extracted straight forwardly from coconut under ambient temperature; therefore, the loss of minor components like pro-vitamin A, vitamin E, and phenolics compounds due to solar UV irradiation during coconut drying can be avoided. VCO may have more beneficial effects than copra oil since it retains most of the unsaponifiable components (Nevin and Rajamohan, 2008). For these reasons, the simultaneous quantitative analysis of both oils (EVOO and VCO) is highly demanded. Consequently, both of oils are target of adulteration with low-priced oils such as palm oil and other vegetable oils (Rohman and Che man, 2009; Rohman and Che Man, 2010).

Various vibrational spectroscopic methods such as infrared (IR) and Raman spectroscopies coupled with chemometrics technique of multivariate calibration have evolved as emerging analytical tool to quantify the oil contents. We have developed Fourier transform infrared (FTIR) spectroscopy in combination with multivariate calibration of partial least square regression (PLSR) and principle component regression (PCR) to analyze palm oil as adulterant in EVOO (Rohman and Che Man,

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2010) and in VCO (Rohman and Che Man, 2009). Unfortunately, using literature searching, there is no available report related to the application of FTIR and chemometrics for quantitave analysis of EVOO and VCO simultaneously; therefore, the objective of this research was to optimize FTIR spectroscopy combined with multivariate calibrations of partial least square and principle component regression (PCR) to simultaneously analyze EVOO and VCO. Furthermore, the developed method can be used to detect the presence of other edible oils having low priced-oils as adulterants, either in VCO, EVOO, or in both oils.

Materials and Methods

Extra virgin olive oil (Selva®) was bought from super market in Selangor Malaysia. Virgin coconut oil used is produced by Department of Food Technology, Universiti Putra Malaysia. Standard fatty acid methyl ester (FAME) was bought from Sigma Aldrich. The solvents and chemicals used in all studies were of pro-analytical (p.a) grade.

Analysis of fatty acid composition

The composition of fatty acid (FA) in EVOO and VCO was analyzed using gas-chromatography (GC) with flame ionization detector (FID) and polar column of RTX-5. The derivatization and GC conditions used for such determination can be seen in our previous paper (Rohman and Che Man, 2009). Fatty acid identification was carried out by comparing the retention time of FA in samples with that of standard FAME. Quantification of FAME was performed using the internal normalization as follows:

$$\text{Percentage of fatty acid } x\ (\%) = \frac{\text{peak area fatty acid } x}{\text{total peak area}} \text{ x } 100\ \%$$

FTIR calibration

A set of thirty calibration samples consisting of pure EVOO, pure VCO, and the mixture of both oils in concentration range of 0-100% was prepared. For predictive capability of the calibration model, twenty eight independent samples were built. All samples are subjected to FTIR spectroscopy measurements.

FTIR spectroscopy

Using Pasteur pipette, a few drops of oil samples were placed on horizontal attenuated total reflectance (HATR) using Smart Attenuated Total Reflectance kit (ARK) (Thermo Electron Corp.) with dimension of 10 x 60 mm composed of ZnSe crystal, producing 12 internal reflections with a penetration depth (infrared beam) of 2.0 µm, with an aperture angle of 45° and refractive index of 2.4 at 1000 cm^{-1}, using FTIR spectrometer from Thermo Nicolet Corp., Madison, WI, equipped with deuterated triglycine sulphate (DTGS) as a detector and potassium bromide (KBr)/ Germanium as beam splitter, and connected to software of the OMNIC operating system (Version 7.0 Thermo Nicolet). FTIR spectra were obtained from 32 scans at a resolution of 4 cm^{-1} with strong apodization throughout the mid infrared region (4000–650 cm^{-1}). These spectra were subtracted against background air spectrum and recorded as absorbance values at each data point in triplicate.

Statistical analysis and validation

Analysis of spectra data using multivariate calibration of partial least square and principle component regression (PCR) was performed with the aid of the software TQ Analyst™ version 6 (Thermo electron Corporation, Madison, WI). The optimum number of principal components (PC) or factors in PLS and PCR was determined by cross validation, employing cancellation one standard in calibration model at a time by plotting the number of factors against the root mean square error of cross validation (RMSECV) and determining the minimum PC. The predictability of the models was tested by computing root mean square error of prediction (RMSEP) as described by Gurdeniz and Ozen (2009).

Results and Discussion

FTIR spectra and Fatty acid profiles

Fatty acid composition and triglyceride profiles were usually exploited as specific components in the analysis of edible fats and oils. However, the problem arises when the purpose of quantification is to analyze the edible oils as a whole matter. For this reason, FTIR spectroscopy is an alternative solution because this technique performed the determination of analytes of interest in whole matter. Figure 1 exhibits FTIR spectra of EVOO and VCO measured in mid infared region of 4000 – 650 cm^{-1} which represent the common spectra of edible oils as described by Safar *et al*. (1994) and Guillen and Cabo (1997). This region corresponds to the vibration of functional groups present in both oils. Basically, fats and oils are constituted from fatty acids esterified with trihydroxy alcohol (glycerol) with different carbon number (chain length), position of double bonds and fatty acids within the molecule of glycerol (O'Brien, 2004).

Upon detail investigation, there are some differences between EVOO and VCO spectra. Peaks at region 3007 cm^{-1} attributed to *cis* –C=CH vibration and at 1654 cm^{-1} caused by vibration of *cis* C=C were

present in EVOO, and otherwise was not observed in VCO. This peak was also correlated with the presence of unsaturated fatty acid. The more unsaturated the fatty acid, the higher the peak intensities at 3007 and 1654 cm^{-1} (Guillen and Cabo, 1997). From Table 1, it can be stated that EVOO has the higher unsaturated fatty acid, especially oleic acid (C18: 1) than VCO. In addition, at spectral regions of 1120 – 1090 cm^{-1}, EVOO revealed two peaks at 1117 and 1098 cm^{-1}, meanwhile VCO only appears one peak at 1116 cm^{-1}. These variations should be optimized for the selection of spectral regions. In this study, FTIR spectra regions at the combined frequencies of 3050 – 3000, 1660 – 1650, and 1200 – 900 cm^{-1} were selected for quantification of EVOO and VCO simultaneously.

Table 1. Fatty acid composition (%) of extra virgin olive oil (EVOO) and virgin coconut oil (VCO)

Fatty acid	EVOO		VCO	
	Sample	Standard Codex	Sample	[b]Standard Codex
C6:0	nd	-	0.06 ± 0.00	nd - 0.70
C8:0	nd	-	7.37 ± 0.22	4.60 - 10.0
C10:0	nd	-	6.62 ± 0.18	5.0 – 8.0
C12:0	nd	-	50.01 ± 1.07	45.10 - 53.20
C14: 0	0.01 ± 0.00	0.0 – 0.05	19.26 ± 0.84	16.80 - 21.00
C16:0	10.88 ±0.42	7.5 -20.0	10.01 ± 0.28	7.50 - 10.20
C16:1	0.77 ± 0.04	0.3 – 3.5	nd	-
C18:0	3.24 ± 0.13	0.5 – 5.0	4.81± 0.32	2.00 - 4.00
C18:1	73.27 ± 0.86	55.0 – 83.0	0.90 ± 0.04	5.0 – 10.0
C18:2	7.06 ± 0.03	3.5 - 21.0	1.05 ± 0.03	2.00 - 4.00
C20:0	0.60 ± 0.00	0.0 – 0.6	nd	-
C18:3	0.33 ± 0.03	< 1.0[a]	0.10 ± 0.01	nd – 0.2
C20:1	0.36 ± 0.05	0.0 – 0.4	nd	-
C22:0	0.13 ±0.01	0.0 – 0.2	nd	-
C24:0	0.10 ± 0.00	0.0 – 0.2	nd	-

[a]taken from IOOC regulation as cited from Piravi-Vanak (2010). [b]for RBD coconut oil; nd = not detected; - = not determined. Each value in the table represents the means of triplicate analysis; SD is given after ±

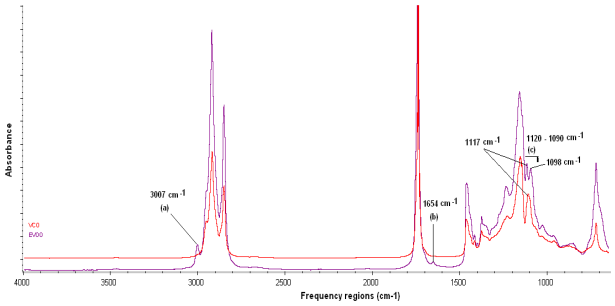

Figure 1. FTIR spectra of extra virgin olive oil (EVOO) and virgin coconut oil at mid infrared region (4000 – 650 cm^{-1}).

Quantitative analysis of EVOO and VCO

Quantitative analysis of EVOO and VCO was performed simultaneously with the aid of multivariate calibration of PLSR and PCR. PLS calibration model works with the information obtained from the whole spectra to develop the regression equation between spectra and concentration of analytes of interest. Meanwhile, PCR performs multiple inverse regressions of the predictor variables against the scores rather than the original data (Romía and Bernàrdez, 2008).

The multivariate calibration of PLS and PCR models were subjected to be optimized by investigating the frequency regions and spectral treatments (either normal or derivatization) in such a way that offers the lowest values of RMSEC and the highest values of R. The first derivative omits the intensity effect encountered in FTIR spectral and can simplify the selection of spectra baseline, while the second derivatization can remove the slope effect. Unfortunately, the derivation treatments can strongly influence the measurement sensitivities. Therefore, the use of derivative FTIR spectra should be avoided if the concentration of analytes of interest is very low (Cadet and de la Guardia, 2001). The next optimization was carried by evaluation of standard normal variate (SNV) treatment which scales FTIR spectral data in order to compensate the pathlength differences (Wang *et al*., 2006).

Table 2 compiled the performance of multivariate calibration of PLSR and PCR for the simultaneous determination of EVOO and VCO using the combination of spectral treatments at the optimized frequencies of 1200- 900, 2827.13 - 2397.08 and 3050 – 3000 cm^{-1}. In general, PLSR with MC treatment (either using normal or derivative spectra) offers the highest value of R and the lowest value of RMSEC and RMSEP compared with other treatments. Beside, these treatments also exploited less the number of principal components or factors in the development of multivariate model; for this reason, PLSR with MC treatment is chosen for quantification of EVOO and VCO. In the specific manner, based on the highest values of R and the lowest values of RMSEC, PLSR with MC and first derivative spectra was selected for such determination. Figure 2 showed the scatter plot for the relationship between actual value and FTIR predicted value of EVOO and VCO in the calibration model using PLSR with mean centered-first derivative spectra.

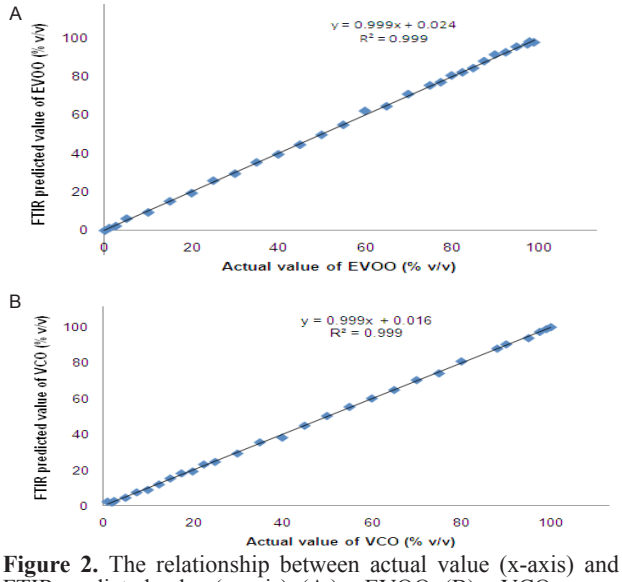

Figure 2. The relationship between actual value (x-axis) and FTIR predicted value (y-axis). (A) = EVOO; (B) = VCO.

Table 2. The performance of PLSR and PCR for simultaneous determination of EVOO and VCO using several spectral treatments

Spectral treatments	Cal	Factor	EVOO			VCO		
			R	RMSEC	RMSEP	R	RMSEC	RMSEP
Normal + MC	PLS	3	0.9987	1.67	2.11	0.9986	1.74	2.19
Normal + MC	PCR	10	0.9988	1.66	2.06	0.9987	1.73	2.13
1st der + MC	PLS	4	0.9977	0.719	2.40	0.9998	0.657	2.47
1st der + MC	PCR	10	0.9985	1.82	2.14	0.9985	1.80	2.16
2nd der + MC	PLS	4	0.9992	1.29	3.81	0.9993	1.26	3.85
2nd der + MC	PCR	10	0.9981	2.03	4.04	0.9983	1.97	4.03
Normal + SNV	PLS	3	0.9938	3.73	4.95	0.9942	3.61	5.09
Normal + SNV	PCR	9	0.9947	3.45	5.11	0.9949	3.40	5.20
1st der + SNV	PLS	5	0.9993	1.18	5.73	0.9994	1.16	5.91
1st der + SNV	PCR	10	0.9948	3.42	6.08	0.9951	3.31	6.15
2nd der + SNV	PLS	4	0.9969	2.62	7.24	0.9971	2.55	7.49
2nd der + SNV	PCR	10	0.9961	2.67	6.16	0.9962	2.92	6.30

MC = mean centering; SNV = standard normal variate; der = derivative.

The capability of calibration model using PLSR was tested to predict the concentration of independent samples. For this purpose, twenty eight samples were prepared. The R and root mean square error of prediction (RMSEP) were used for the validity criteria. Table 3 lists the R, RMSEP and equation obtained for the quantification of EVOO and VCO. It can be stated that PLSR using mean-centered first derivative spectra was appropriate for such determination due to the ability to predict the independent samples with acceptable R and RMSEP values.

Table 3. The values of R and RMSEP values together with the equation obtained for the prediction of EVOO and VCO

Sample	R	RMSEP	Equation
EVOO	0.9977	2.40	y = 1.020x – 0.238
VCO	0.9998	2.47	y =1.013x – 1.152

In order to validate the calibration model of PLSR, a cross validation technique using one-leave-out technique was used. In this technique, the first sample is removed from the calibration data set and the remaining samples (sample 2 – 30) are used to find the regression model. Subsequently, the omitted sample (first sample) is predicted using the new regression. This procedure was repeated, leaving each specimen out in turn. Then, for each calibration sample, the difference between actual and predicted value is calculated. The sum of the square of the discrepancies is named with predicted residual error of sum of squares (PRESS). The lower the PRESS value, the better the predictive capability of the developed model (Miller and Miller, 2005).

Figure 3 revealed the correlation between root mean square error of cross validation (RMSECV) and PRESS. The RMSECV values obtained for quantification of EVOO and VCO are 3.217 and 2.94 % (v/v), respectively. From PRESS values in Fig. 3, it can be stated that the optimal principal components (PCs) or factors is 4, because RMSECV obtains a stable value, minimally after four-factor. The optimum number of PCs in PLSR corresponds to the point at which the PRESS plot reaches a minimum or begins to level off (Sedman *et al*. 1997). Based on the result, it can be stated that EVOO and VCO can be simultaneously determined using FTIR spectroscopy in combination with chemometrics of multivariate calibration. Furthermore, the developed method can also be extended to analyze the presence of other oils as adulterants in EVOO and VCO.

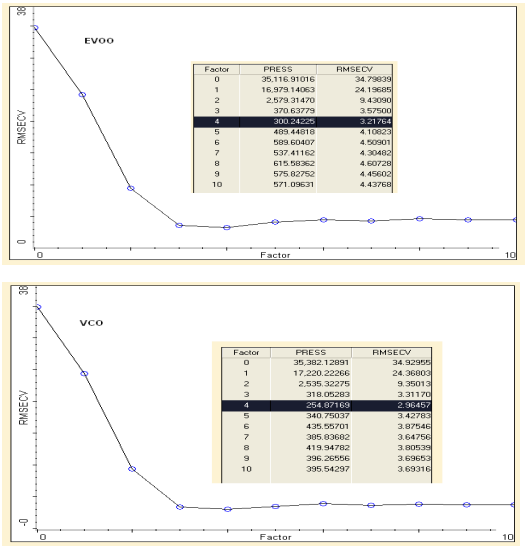

Figure 3. The correlation between root mean square error of cross validation (RMSECV) and predicted residual error sum squares (PRESS) for determination of EVOO (A) and VCO (B).

Conclusion

From the above results, it can be deduced that FTIR spectroscopy, as one of the fingerprint technique can be a potential technique for the simultaneous analysis of EVOO and VCO in the mixtures. FTIR spectroscopy offers some advantages, namely fast, ease of use in instrumental operation, and no excessive sample preparation. Besides, the use of hazardous solvents and reagents can be avoided; therefore, the use of FTIR spectroscopy as an analytical technique for edible oil analysis can promote "the green analytical technique".

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