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ORIGINAL PAPER

Application of FTIR Spectroscopy for the Determination of Virgin Coconut Oil in Binary Mixtures with Olive Oil and Palm Oil

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Abstract Rapid Fourier transform infrared (FTIR) spectroscopy combined with attenuated total reflectance (ATR) was applied for quantitative analysis of virgin coconut oil (VCO) in binary mixtures with olive oil (OO) and palm oil (PO). The spectral bands correlated with VCO, OO, PO; blends of VCO and OO; VCO and PO were scanned, interpreted, and identified. Two multivariate calibration methods, partial least square (PLS) and principal component regression (PCR), were used to construct the calibration models that correlate between actual and FTIR-predicted values of VCO contents in the mixtures at the FTIR spectral frequencies of 1,120–1,105 and 965–960 cm^{-1}. The calibration models obtained were cross validated using the "leave one out" method. PLS at these frequencies showed the best calibration model, in terms of the highest coefficient of determination (R^2) and the lowest of root mean standard error of calibration (RMSEC) with $R^2 = 0.9992$ and RMSEC = 0.756, respectively, for VCO in mixture with OO. Meanwhile, the R^2 and RMSEC values obtained for VCO in mixture with PO were 0.9996 and 0.494, respectively. In general, FTIR spectroscopy serves as a suitable technique for determination of VCO in mixture with the other oils.

Keywords FTIR · Virgin coconut oil · Binary mixture · Partial least square · Principal component regression

Introduction

As a relative newcomer in the fat and oil markets, virgin coconut oil (VCO) is rapidly becoming a valuable oil which can be compared with olive oil. The demand of this oil is continuing, which can be attributed not only to its superior flavor, but also to the reports regarding its potential health benefits [1]. VCO is derived from fresh coconuts and is directly extracted from coconut meat under mild temperatures. This extraction process avoids the loss of minor components like vitamin E, pro-vitamin A, and polyphenols, due to UV irradiation from sunlight during drying of copra. VCO may exhibit more beneficial effects than copra oil, since it retains most of the unsaponifiable components [2]. VCO contains a significant amount of medium-chain fatty acids, such as capric, caproic and caprylic, which were also investigated to show antimicrobial and antiviral activities [3].

Several researches have been carried out on VCO related to its biological activities. Nevin and Rajamohan [4] reported that VCO lowered the levels of total cholesterol, triglycerides, phospholipids, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterols, and increased high density lipoprotein (HDL) cholesterol in the serum and tissues. They also found that the polyphenol fraction of VCO was capable of preventing

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LDL oxidation in vitro with the formation of reduced carbonyl. Moreover, administration of VCO can enhance the antioxidant enzymes and reduces the lipid peroxidation content [5]. VCO also has a more significant antithrombotic effect compared with copra oil [2]. Because of its biological effects, an analytical method offering fast and reliable for quantitative analysis of VCO should be developed.

Analysis of VCO is usually performed by determination of specific components present in VCO such as fatty acid methyl esters (FAME) using gas chromatography and triglyceride compositions with liquid chromatography (1) rather than analysis of VCO as a whole matter. For this reason, it is proposed that FTIR can be used as an analytical method to overcome such problems. Infrared spectroscopy is a technique which can be easily used by untrained personnel in some laboratories. A particular attraction is the ease of the sample presentation [6].

In recent years, because of the development of Fourier transform infrared (FTIR) spectroscopic instrumentation, the application of this technique has become popular in the study of edible oils and fats [7]. The importance of IR spectroscopy for identification of molecular structures comes from the large information content obtained and the possibility to assign certain absorption bands, in which most of the peaks and shoulders in fats and oils are attributable to the specific functional groups [8].

Multivariate calibration is one of the useful chemometrics techniques frequently employed for analysis of complex mixtures [9]. Partial least square (PLS) and principal component regression (PCR) are commonly used multivariate calibration methods in the analysis of fats and oils, based on data reduction and inverse calibration [10]. PLS enables one to use information of sample spectra from wide spectral frequencies [11]. Meanwhile, PCR is a type of factor analysis, where the spectral and concentration data are included into the model in one step [12]. Multivariate calibration methods can provide information related to method precision and yield important qualitative and diagnostic information. Thus, they can provide better accuracy, precision, reliability and saving time than previous data analysis methods [13].

Currently, because of software advances, multivariate statistical techniques have been successfully used by FTIR spectroscopists for quantitative analysis purposes [14]. Combined with multivariate calibrations, FTIR spectroscopy has been reported for the analysis of certain oils in the mixture with others for authentication issues such as canola, walnut, sunflower, soybean, peanut, corn, and sesame oils in olive oil [15], hazelnut oil in extra virgin olive oil (EVOO) [16], hazelnut oil in refined olive oil [17], soybean oil in camellia oil [18], sunflower, corn, soybean and hazelnut oils in EVOO [19], and lard in cod-liver oil [20]. However, a little information is available regarding the analysis of VCO using FTIR spectroscopy compared with other oils like olive oil.

Our group has used FTIR spectroscopy combined with multivariate calibration of PLS and discriminant analysis to analyze palm kernel oil as an adulterant in VCO [21]. There is no previous report regarding analysis of VCO in a mixture with other oils. Therefore, the objective of the research was to develop a fast FTIR spectroscopic technique for determination of VCO in the binary mixture of oils, namely PO and OO as the mixture models, using multivariate calibration techniques.

Materials and Methods

Virgin coconut oil (POVCO®), palm oil (Bimoli®), and extra virgin olive oil (Bertolli®), were purchased from a local market in Jogjakarta, Indonesia.

Calibration and Validation Standard

For the construction of a calibration curve, a 27 data set of VCO was mixed with OO or PO in ratios of 1–50% wt/wt in chloroform. The mixture was shaken vigorously to ensure complete homogenization. For validation, 38 independent samples were constructed. All samples were analyzed using the FTIR spectrometer. The spectral regions where the variations were observed were chosen for developing PLS and PCR models.

FTIR Spectroscopic Analysis of VCO

Using a Pasteur pipette, a few drops of each sample were placed in contact with attenuated total reflectance (ATR) on a multibounce plate of ZnSe crystal at a controlled ambient temperature (25 °C). A Nicolet 6700 FTIR spectrometer (Thermo Nicolet Corp., Madison, WI, USA) equipped with a detector of deuterated triglycine sulfate (DTGS), a KBr/Germanium beam splitter, and connected to software of the OMNIC operating system (Version 7.0 Thermo Nicolet), was used during FTIR data acquisition. To minimize interferences caused by water vapor, the FTIR spectrometer was maintained with the dehumidifier composed of silica gel.

The collection of FTIR spectra was carried out at 32 scans with resolution of 4 cm^{-1} using strong apodization in the frequency regions of 4,000–650 cm^{-1}. These spectra were subtracted against an air spectrum (background). After every scan, a new reference air background spectrum was taken. The ATR plate was carefully cleaned in situ using hexane twice followed by acetone and dried with a soft tissue before filling with the next sample. Cleanliness was verified by observing a background spectrum and comparing it to the previous one. These spectra were recorded as absorbance values, and replication was done three times.

Statistical Analysis and Validation

Chemometrics analysis, including PLS and PCR was performed using the software of TQ Analyst^{TM} version 6 (Thermo electron Corporation, Madison, WI, USA). The "leave-one-out" cross-validation procedure was used to verify the calibration model. Validity criteria for the calibration were evaluated by computing the values of the root mean standard error of calibration (RMSEC) and coefficient of determination (R^2). The validation was further investigated using the mean difference (MD) and standard deviation of difference (SDD) for accuracy and reproducibility.

Results and Discussion

Spectra Analysis

Triglycerides are the principal component in fats and oils and, consequently, dominate FTR spectra of fats and oils. Figure 1 shows the FTIR spectra of VCO, olive oil and palm oil. These spectra look very similar and showed a typical characteristic of absorption bands for common triglyceride [22]. The analytical evaluation of the VCO spectrum in term of functional groups corresponding for absorption of certain frequencies is given in Table 1.

Calibration and Validation

VCO in Mixture with Olive oil

Figure 2 shows the FTIR spectra of VCO in a mixture with OO in the range of 0–50% VCO in OO at wavenumbers 4,000–650 cm^{-1}. The difference between the VCO spectrum and the OO spectrum can be seen in the frequency region of 1,111 cm^{-1} (a) and 962 cm^{-1} (b), which is illustrated in Fig. 2. Furthermore, these frequencies were employed for making the calibration and validation models, either using PLS or PCR techniques.

Table 1 Functional groups and modes of vibration in VCO spectra

Frequency (cm^{-1})	Functional group	Vibration modes
2,954	–C–H (–CH_3)	Stretching
2,924	–C–H (–CH_2)	Asymmetric stretching
2,852	–C–H (–CH_2)	Symmetric stretching
1,743	–C=O (Carbonyl ester)	Stretching
1,465	–C–H (–CH_2)	Bending (scissoring)
1,417	=C–H (*cis*)	Bending (scissoring)
1,377	–C–H (–CH_3)	Symmetrical bending
1,228	–C–O	Stretching
1,155	–C–O	Stretching
	–CH_2–	Bending
1,111	–C–O	Stretching
962	–HC=CH– (*trans*-olefin)	Bending (out of plane)
872	=CH_2	Bending (wagging)
721	–$(CH_2)n$–	Bending (rocking)
	–HC=CH– (*cis*-olefin)	

Taken from Refs. [23] and [24]

In the PLS and PCR calibration models, evaluation of the method linearity was performed in order to demonstrate the proportional relationship between responses (absorbances) versus analyte concentration of VCO in the mixture over the working range.

The R^2 value and RMSEC value from the PLS and PCR calibrations were used to choose the best region for determining VCO in the mixture in the spectral ranges of 1,120–1,105 cm^{-1} and 965–960 cm^{-1}. The results obtained from the PLS and PCR calibrations in terms of R^2 and RMSEC are shown in Table 2.

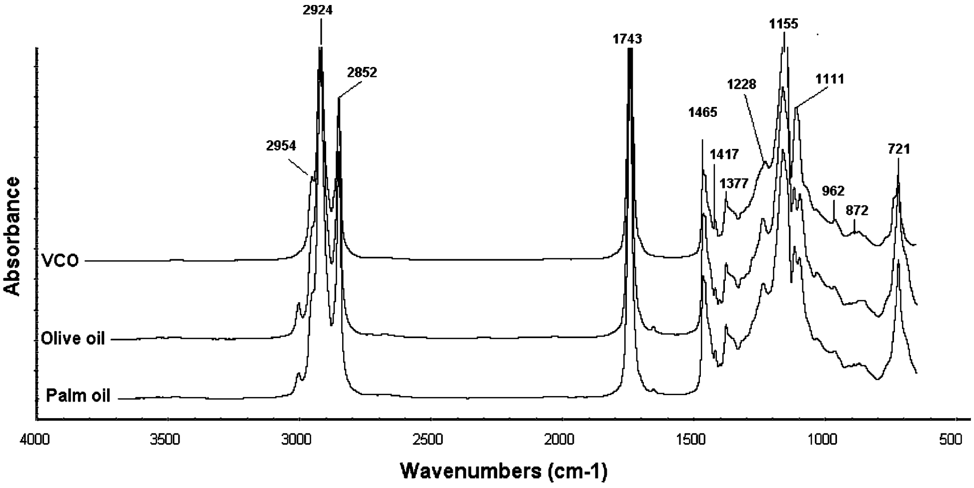

Fig. 1 FTIR spectra of virgin coconut oil (VCO), palm oil (PO), and olive oil (OO) at wavenumber of 4,000–650 cm^{-1}

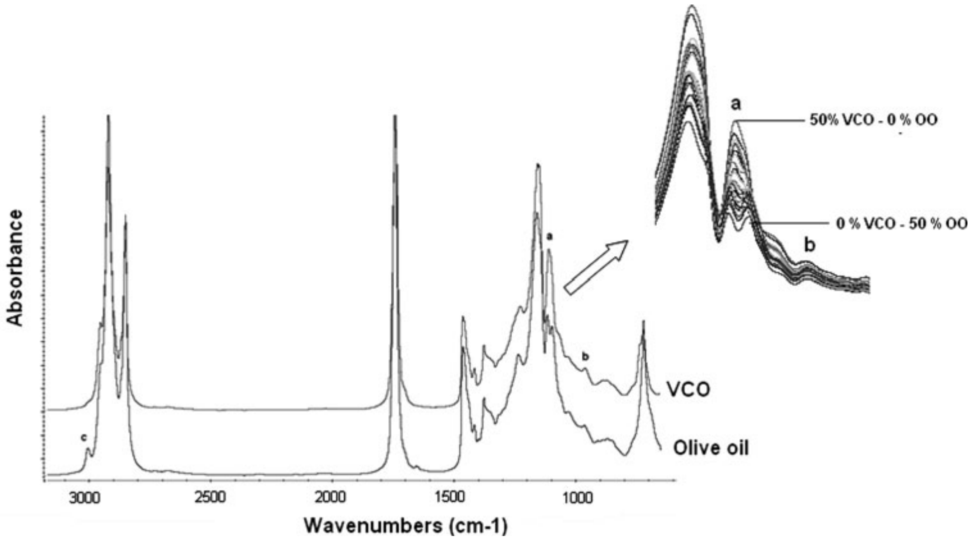

Fig. 2 FTIR spectra of VCO in mixture with OO in the range of 0–50% VCO in OO at wavenumbers of 4,000–650 cm^{-1}

Table 2 Calibration statistics for determining the VCO content in olive oil using PLS and PCR techniques

Spectral ranges (cm^{-1})	PLS		PCR	
	R^2	RMSEC	R^2	RMSEC
1,120–1,105	0.9991	0.761	0.9723	4.03
965–960	0.9571	4.98	0.8612	8.69
1,120–1,105 and 965–960	0.9992	0.756	0.9991	0.758

For comparison between PLS and PCR used, PLS at two frequency regions of 1,120–1105 and 965–960 cm^{-1} revealed the highest of R^2 and the lowest of RMSEC compared with frequency at region 1,120–1,105 cm^{-1} and frequency region of 965–960 cm^{-1} (Table 2). Using PLS at frequency of 1,120–1,105 and 965–960 cm^{-1} for calibration model, the R^2 of 0.9992 and RMSEC of 0.756 were obtained for the relationship between actual value and FTIR predicted value of VCO contents (% wt/wt). Therefore, these frequency regions (1,120–1,105 and 965–960 cm^{-1}) were preferable for use for the prediction of VCO content in the mixture with olive oil, in terms of the highest R^2 value and the lowest of RMSEC value.

In order to validate the developed model, cross validation using the 'leave one out' technique was used. Cross-validation evaluates the data by excluding selected samples in the regression model and then developing a model for the residual samples. The model is evaluated using the samples excluded from the model and followed by computing the error values for the predicted observations. The new samples are then excluded from the model set and a new model is developed. This procedure is performed repeatedly until all samples in the PLS model have been excluded once [25]. After predicting all the observations once using cross-validation technique, the R^2 and RMSEP values were computed for the relationship between actual VCO value and FTIR predicted value at two frequency regions, namely 1,120–1,105 and 965–960 cm^{-1}. The R^2 and RMSEP values were employed to evaluate the goodness of fit of the validation data set, which were calculated with respect to the results obtained from cross validation. The R^2 and RMSEP values obtained were 0.997 and 0.823, respectively. Both high value of R^2 and the low value of RMSEP indicate the success of the PLS calibration model. The PLS regression model appears to have a reasonable ability to estimate the VCO percentage in the mixture with OO, based on the high R^2 and low RMSEP values.

VCO in a Mixture with Palm Oil

FTIR spectra of VCO in a mixture with palm oil (PO) were shown in Fig. 3. The differences between VCO and PO can be seen at frequency regions of 1,120–1,105 cm^{-1} (a) and 965–960 cm^{-1} (b), as shown in Fig. 3. These frequencies were employed for quantitative analysis of VCO in a mixture with PO.

The PLS and PCR calibration techniques were employed for making the calibration models in the determination of VCO in a mixture with PO in the spectral ranges of 1,120–1,105 cm^{-1} and 965–960 cm^{-1}. The values of R^2 and RMSEC from the PLS and PCR calibration were further used to choose the best region for determining VCO in a mixture with PO. The results obtained are shown in Table 3.

It can be shown that the PLS calibration technique at two frequencies of 1,120–1,105 and 965–960 cm^{-1} is the better model, in terms of the highest value of R^2 (0.9996) and the lowest value of RMSEC (0.494) for the relationship between the actual value (x-axis) and the FTIR predicted (y-axis) VCO contents with the equation of $y = 0.999x + 0.020$.

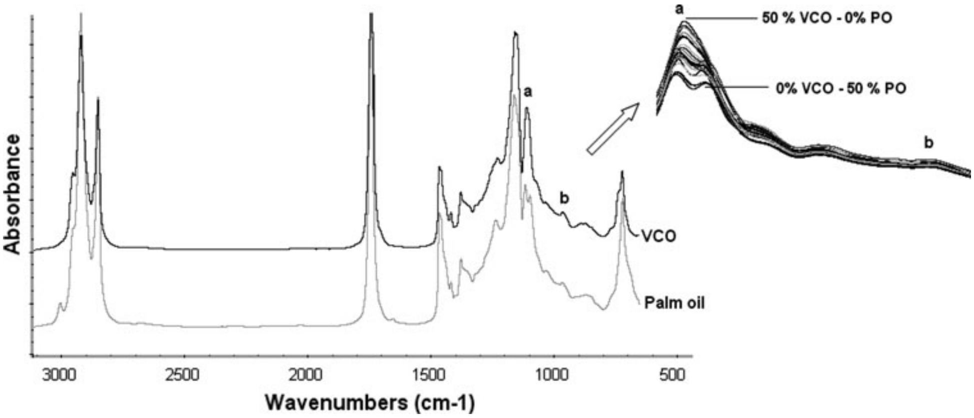

Fig. 3 FTIR spectra of VCO in a mixture with PO in the range 0–50% of VCO in PO at wavenumbers of 4,000–650 cm^{-1}

Table 3 Calibration statistics for determining of VCO content in palm oil using PLS and PCR techniques

Spectral ranges	PLS		PCR	
	R^2	RMSEC	R^2	RMSEC
1,120–1,105	0.9995	0.529	0.9750	3.80
965–960	0.8309	9.54	0.8308	9.52
1,120–1,105 and 965–960	0.9996	0.494	0.9742	3.86

The PLS regression model appears to have a reasonable ability to estimate the VCO percentage in the mixture with PO, based on the high R^2 and low RMSEC.

The cross validation showed that the R^2 and RMSEP values obtained were 0.997 and 0.823, respectively. The high value of R^2 and the low value of RMSEP indicate that the PLS calibration model was suitable for determining VCO in the mixture with PO. The equation obtained in the validation model for the relationship between the actual value and the FTIR predicted value of VCO contents in the mixture with PO is $y = 0.992x + 0.096$.

Table 4 shows the statistical results calculated from cross validation as mean difference (MD) and standard deviation of difference (SDD) for accuracy and reproducibility for the determination of VCO in mixture with OO and PO.

Table 4 Repeatability and accuracy of FTIR predicted values of the VCO content in mixtures with OO and PO obtained by cross validation

Statistics	OO		PO	
	Actual	FTIR	Actual	FTIR
MD*a*	0.050		0.083	
SDD*a*	0.833		0.802	
MD*r*		0.062		0.095
SDD*r*		0.907		0.964

MD mean difference; *SDDr* standard deviation of difference; *a* accuracy; *r* repeatability

The term accuracy refers to the closeness of agreement between the actual data and the predicted data of FTIR results. The low value of MDa (0.050 and 0.083), and SDDa (0.833 and 0.802) for VCO in mixtures with OO and PO, respectively, indicated that the FTIR is an appropriate analytical technique for quantitative analysis of VCO in mixture with OO and PO. Meanwhile, low MDr (0.062 and 0.095) and SDDr (0.907 and 0.964), indicated that the FTIR method is precise enough.

Conclusions

We concluded that Fourier Transform Infrared (FTIR) spectroscopy combined with ATR and multivariate calibrations of PLS and PCR at frequency regions of 1,120–1,105 and 965–960 cm^{-1} can be used to analyze the VCO contents in mixtures with OO and PO. The method developed was rapid; with a total analysis time of about 3 min for each measurement, and it is environmentally friendly. Furthermore, it is not tediously time consuming and large amounts of chemical reagents and solvents can be avoided. This method can be extended to be applied in the analysis of VCO as an adulterant in more expensive oils such as olive oil.

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References

1. Marina AM, Che Man YB, Nazimah SAH, Amin I (2009) Chemical properties of virgin coconut oil. J Am Oil Chem Soc 86:301–307
2. Nevin KG, Rajamohan T (2008) Influence of virgin coconut oil on blood coagulation factors, lipid levels and LDL oxidation in cholesterol fed Sprague–Dawley rats. Eur J Clin Nutr Met 3:1–8
3. Villarino BJ, Marsha DL, Concepcion M, Lizada C (2007) Descriptive sensory evaluation of virgin coconut oil and refined, bleached and deodorized coconut oil. Lebensm Wiss Technol 40:193–199

4. Nevin KG, Rajamohan T (2004) Beneficial effects of virgin coconut oil on lipid parameters and in vitro LDL oxidation. Clin Biochem 37:830–835
5. Nevin KG, Rajamohan T (2006) Virgin coconut oil supplemented diet increased the antioxidant status in rats. Food Chem 99:260–266
6. Marigheto NA, Kemsley EK, Defernez M, Wilson RH (1998) A comparison of mid-infrared and Raman spectroscopies for the authentication of edible oils. J Am Oil Chem Soc 75:987–992
7. Guillen MD, Cabo N (2000) Some of the most significant changes in the Fourier transform infrared spectra of edible oils under oxidative conditions. J Sci Food Agric 80:2028–2036
8. Bendini A, Cerretani L, Di Virgilio F, Belloni P, Bonoli-Carbognin M, Lercker G (2007) Preliminary evaluation of the application of the FTIR spectroscopy to control the geographic origin and quality of virgin olive oils. J Food Qual 30:424–437
9. Maggio RM, Kaufman TS, De Carlo M, Cerretani L, Bendini A, Cichelli A, Compagnone D (2009) Monitoring of fatty acid composition in virgin olive oil by Fourier transformed infra red (FTIR) spectroscopy. Food Chem 114:1549–1554
10. Paradkar MM, Sivakesava S, Irudayaraj J (2002) Discrimination and classification of adulterants in maple syrup with the use of infrared spectroscopic techniques. J Sci Food Technol 82:497–504
11. Che Man YB, Syahariza ZA, Mirghani MES, Jinap S, Bakar J (2005) Analysis of potential lard adulteration in chocolate and chocolate products using Fourier transform infrared spectroscopy. Food Chem 90:815–819
12. Smith BC (2002) Quantitative spectroscopy: theory and practice. Academic Press, Amsterdam, pp 125–179
13. Setiowaty G, Che Man YB (2003) A rapid Fourier transform infrared spectroscopic method for the determination of 2-TBARS in palm olein. Food Chem 81:147–154
14. Haaland DM, Thomas EV (1988) Partial least squares methods for spectral analysis. I. Relation to other quantitative calibration methods and the extraction of qualitative information. Anal Chem 60:1193–1202
15. Tay A, Singh RK, Krishnan SS, Gore JP (2002) Authentication of olive oil adulterated with vegetable oils using Fourier transform infrared spectroscopy. Lebensm Wiss Technol 35:99–103
16. Ozen BF, Mauer LJ (2002) Detection of hazelnut oil adulteration using FT-IR spectroscopy. J Agric Food Chem 50:3898–3901
17. Baeten V, Fernández-Pierna A, Dardenne P, Meurens M, García-González DL, Aparicio-Ruiz R (2005) Detection of the presence of hazelnut oil in olive oil by FT-Raman and FT-MIR spectroscopy. J Agric Food Chem 53:6201–6206
18. Wang L, Lee FSC, Wang X, He Y (2006) Feasibility study of quantifying and discriminating soybean oil adulteration in camellia oils by attenuated total reflectance MIR and fiber optic diffuse reflectance NIR. Food Chem 95:529–536
19. Lerma-García MJ, Ramis-Ramos G, Herrero-Martínez JM, Simó-Alfonso EF (2010) Authentication of extra virgin olive oils by Fourier-transform infrared spectroscopy. Food Chem 118:78–83
20. Rohman A, Che Man YB (2009) Analysis of cod-liver oil adulteration using Fourier transform infrared (FTIR) spectroscopy. J Am Oil Chem Soc 86:1149–1153
21. Manaf MA, Che Man YB, Hamid NSA, Ismail A, Syahariza ZA (2007) Analysis of adulteration of virgin coconut oil by palm kernel olein using Fourier transform infrared spectroscopy. J Food Lipids 14:111–121
22. Safar M, Bertrand D, Rober P, Devaux MF, Genot C (1994) Characterization of edible oils, butter and margarines by Fourier transform infrared spectroscopy with attenuated total reflectance. J Am Oil Chem Soc 71:371–377
23. Guillen MD, Cabo N (1997) Characterization of edible oils and lard by Fourier transform infrared spectroscopy. Relationships between composition and frequency of concrete bands in the fingerprint region. J Am Oil Chem Soc 74:1281–1286
24. Vlachos N, Skopelitis Y, Psaroudaki M, Konstantinidou V, Chatzilazarou A, Tegou E (2006) Applications of Fourier transform-infrared spectroscopy to edible oils. Anal Chim Acta 573–574:459–465
25. Gurdeniz G, Tokatli F, Ozen B (2007) Differentiation of mixtures of monovarietal olive oils by mid-infrared spectroscopy and chemometrics. Eur J Lipid Sci Technol 109:1194–1202