

Simultaneous quantitative analysis of red fruit oil and sesame oil using FTIR spectroscopy and multivariate calibrations

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Abstract

Sesame oil (SeO) and red fruit oil (RFO) share several similarities, especially in color properties. Both SeO and RFO are red in color. The objective of this study was to analyze SeO and RFO simultaneously using FTIR spectroscopy and multivariate calibrations of partial least square and principle component regressions. Some frequency regions and spectral treatments were optimized. The selection of the spectral treatment, frequency region and multivariate calibrations types was based on the ability to provide the highest values of coefficient of determination (R^2) and the lowest values of errors, either in calibration or prediction. Based on these criteria, FTIR spectra using normal mode and partial least square, regression at frequency regions of 1200 – 1000 cm^{-1} was suitable for determination of SeO and RFO simultaneously. The developed method is rapid at one minute per sample, sensitive, and considered as a green analytical technique since the use of chemicals and reagents are minimized.

Keywords

FTIR spectroscopy

sesame oil

red fruit oil

multivariate calibrations

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Introduction

Red fruit (*Pandanus conoideus* Lam) is an important fruit mainly grown in Papua province of Indonesia. This fruit is consumed daily as diet. The extract of red fruit and its oil are believed to be functional oil due to its capability to provide some biological activities such as antioxidant (Rohman *et al.*, 2010) and anti-carcinogenic activity. This oil is red in color which is similar to that of sesame oil. Several active compounds have been identified in red fruit oil such as beta-carotene and tocopherol (Rohman *et al.*, 2012). In addition, in the local market, red fruit oil has higher priced-value than common plant oils like palm, soybean, and corn oils. Consequently, RFO can be target of adulteration with those plant oils (Rohman *et al.*, 2011).

Since human civilization, sesame seed and its oil have been utilized as an important food components due to the beneficial effects to human health such as antioxidants (Park *et al.*, 2010a ; Elleuch *et al.*, 2012), lowering platelet aggregation (Reena *et al.*, 2010), preventing atherosclerosis in LDL knockout mice (Bhaskaran *et al.*, 2006), and reducing serum cholesterol (Chen *et al.*, 2005). Namiki (2007) has reviewed several nutraceutical aspects of sesame

oil. Sesame has several active compounds like tocopherols, phytosterols, resveratrol, lignans, and flavonoids (Lee *et al.*, 2009) contributing to above biological activities.

Several instrumental techniques have been used for analysis of sesame oil (SeO), especially for detection and quantification of adulterants in SeO. Such methods are gas chromatography and high performance liquid chromatography through analyses of fatty acid and triacylglycerol compositions respectively (Lee *et al.*, 2001; Park *et al.*, 2010b), liquid chromatography-mass spectrometry (LC-MS) (Wang *et al.*, 2009), electronic nose (Hai and Wang, 2006). These methods are time consuming and requiring skillful operators. Besides, the above methods generally determined specific components present in SeO rather than analysis of SeO and RFO as a whole matter. FTIR spectroscopy in combination with chemometrics techniques provide some advantageous in term of analysis of sample as a whole. Therefore, in this study, FTIR spectroscopy coupled with chemometrics of multivariate calibrations was explored for simultaneous determination of SeO and RFO.

In recent years, FTIR spectroscopy has been proven to be a powerful technique for determination

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of edible oils, as indicated by several publications. Recently, Rohman and Che Man (2012) have reviewed the application of FTIR spectroscopy combined with chemometrics for quantitative analysis of plant oils and animal fats present in other oil. The uses of FTIR spectroscopy in combination with multivariate calibrations for simultaneous analysis could be elaborated to analyze two functional food oils namely extra virgin olive oil and virgin coconut oil (Rohman and Che Man, 2011a). However, using literature searching there is no report available in relation to application of this technique for analysis of SeO and RFO simultaneously. The developed method can be extended for purity determination of SeO and RFO may be subjected to be adulterated with other oils.

Materials and Methods

Materials

Red fruit oil was obtained from red fruit which collected from Papua Province, Indonesia, extracted by liquid-liquid extraction using hexane as oil extracting solvent. Red fruit was slashed into small pieces using a conventional cutter and subsequently subjected to commercial blender containing ethanol. The extracts were further macerated by methanol for 4 days and partitioned using hexane. The hexane extracts containing red fruit oil were evaporated at 60°C until the hexane residue is removed. The oil obtained was further used for analysis of FTIR spectra and fatty acid composition. Sesame oil (SeO) was bought from super market near Yogyakarta. SeO used for this study was the mixture of three commercial oils in order to compensate any variation preset among oils. Standard of fatty acid methyl esters was purchased from Sigma (Allrich, USA). Solvents and reagents used were of pro analytical grade bought from E. Merck (Darmstat, Germany).

Fatty acid analysis

The fatty acid composition of red fruit oil and sesame oil was determined using gas chromatography coupled with flame ionization detector operated at 200 °C. For qualitative analysis, the retention time of each fatty acids was compared to that in standard fatty acid methyl esters (FAME). Quantitative analysis of each fatty acids was performed with the internal normalization technique, as described in Rohman and Che Man (2011b). The column, carrier gas and other conditions of gas chromatograph were adjusted to similar with in our previous report (Rohman *et al.*, 2012).

FTIR Analysis

For calibration samples, 33 samples of red fruit oil

(RFO) in the mixture with sesame oil (SeO) was made. RFO was mixed with SeO in the concentration level of 0 – 100 %. For validation samples, 29 samples of RFO mixed with SeO was also made. FTIR spectra of all samples i.e. pure SeO, pure RFO, and the mixture of both oils with different concentrations were scanned using a FTIR spectrometer ABB MB3000 FTIR spectrometer (Clairet Scientific, Northampton, UK) equipped with detector of deuterated triglycine sulphate (DTGS) and beam splitter of Germanium on KBr substrat. FTIR spectra were scanned using a resolution of 4 cm⁻¹, number of scanning of 32 in the wavenumbers of 400–4000 cm⁻¹. Spectra were analyzed with the software of Horizon MB[®] version 3.0.13.1 (ABB, Canada). The samples were placed in direct contact with ZnSe crystal of attenuated total reflectance at controlled room temperature (20°C).

Data analysis

Quantitative analysis of RFO and SeO was carried using partial least square (PLS) and principle component regression (PCR) using spectral absorbance as predictor. PLS and PCR were done using Horizon MB FTIR software version 3.0.13.1 (ABB, Canada). The leave-one-out cross-validation procedure was used to verify the calibration model. The values of root mean square error of calibration (RMSEC) and coefficient of determination (R²) were used as the validity criteria for calibration model. The predictive ability of PLS calibration model was further used to calculate the validation samples.

Results and Discussion

Fatty acid composition of red fruit oil and sesame oil as determined using gas chromatography was shown in table 1. Oleic acid is the main components composed of red fruit oil, while oleic and linoleic (C18:2) acids are major fatty acids composed of sesame oil. The specification of the used of sesame oil in this study was in agreement with that in Codex, as shown in the similar profile of fatty acids composed of the used sesame oil with that specified in Codex Alimentarius ranges (2001).

FTIR spectra in mid infrared region (4000 – 650 cm⁻¹) of red fruit oil and sesame oil were shown in Figure 1. Both spectra exhibited FTIR spectral

Table 1. Fatty acid composition of red fruit oil and sesame oil

Fatty acids	Fatty acid composition (%)		
	Red fruit oil	Sesame oil	Sesame oil in Codex
Myristic acid (C14:0)	0.08 ± 0.01	0.02 ± 0.00	nd-0.1
palmitic acid (C16:0)	20.08 ± 0.07	10.00 ± 0.07	7.9–12
Palmitoleic acid (C16:1)	0.15 ± 0.01	0.14 ± 0.00	0.1–0.12
Stearic acid (C18:0)	0.17 ± 0.01	7.27 ± 1.09	4.8–6.1
Oleic acid (C18:1)	67.90 ± 0.13	33.69 ± 0.12	35.9–42.3
Linoleic acid (C18:2)	8.95 ± 0.14	46.95 ± 0.16	41.5–47.9
Eicosanoic acid (C20:0)	0.16 ± 0.00	0.36 ± 0.00	0.3–0.6
C18:3 (linolenic acid)	0.14 ± 0.01	0.64 ± 0.01	0.3–0.4
Eicosanoic acid (C20:1)	0.02 ± 0.00	0.15 ± 0.00	0.1–0.5

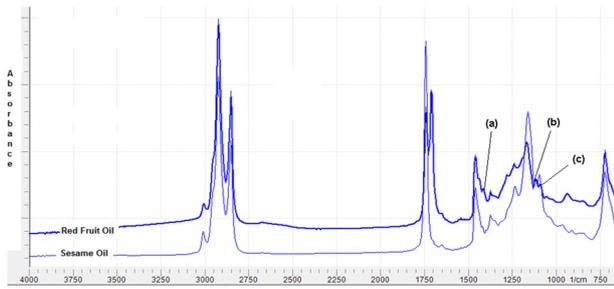


Figure 1. FTIR spectra of red fruit oil and sesame oil at mid infrared region (4000 – 650 cm^{-1})

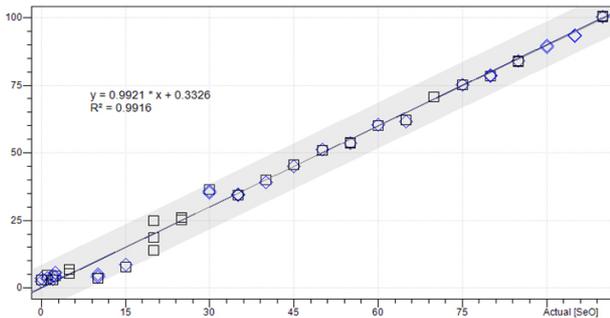


Figure 2. The relationship between actual and FTIR predicted value of sesame oil in PLS calibration model at frequency region of 1200 – 1000 cm^{-1}

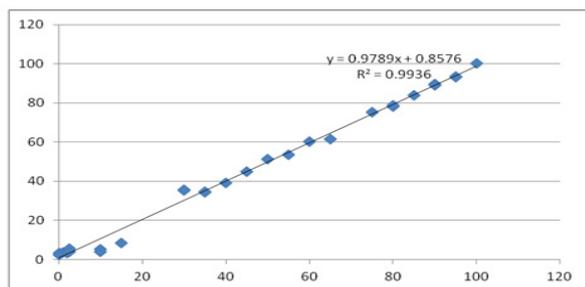


Figure 3. The relationship between actual and FTIR predicted value of sesame oil in PLS prediction model at frequency region of 1200 – 1000 cm^{-1}

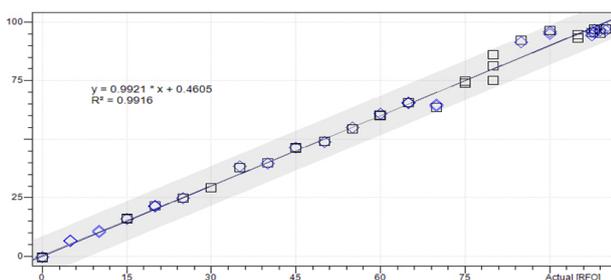


Figure 4. The relationship between actual and FTIR predicted value of red fruit oil in PLS calibration model at frequency region of 1200 – 1000 cm^{-1}

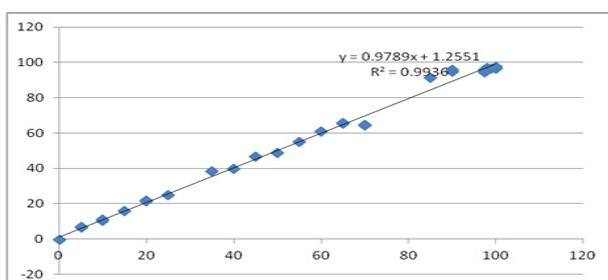


Figure 5. The relationship between actual and FTIR predicted value of red fruit oil in PLS prediction model at frequency region of 1200 – 1000 cm^{-1}

characteristics of edible fats and oils. For qualitative analysis, FTIR spectra can be potential means for differentiation of edible fats and oils. This based on fact that FTIR spectra are taken into account as fingerprint spectra meaning that no two fats and oils having the same FTIR spectra in terms of the sum of peak and shoulder, the intensities of each peaks and shoulders, and the exact frequencies of maximum absorptions either in shoulder or in peak (Vlachos *et al.*, 2006).

Both spectra revealed same profiles in terms of the number of peaks; however, using detailed investigation both oils can be distinguished from its FTIR spectra, especially in fingerprint region (1500 – 650 cm^{-1}). FTIR spectra of RFO can be differentiated from sesame oil in which in which the peak intensities at frequency regions of 1413 cm^{-1} (a), 1117 cm^{-1} (b), and 1097 cm^{-1} (c) were slightly different. Peak at 1416 cm^{-1} corresponds to Rocking vibrations of CH bonds of cis-disubstituted olefins; meanwhile frequencies of 1118 and 1097 cm^{-1} were coming from ether linkage absorptions in TAG (Rohman and Che Man, 2010). The functional groups responsible to the peak and shoulder absorptions in FTIR spectra of red fruit oil and sesame oil can be seen in our previous reports (Rohman *et al.*, 2011; Rohman and Che Man, 2011b). The variations of FTIR spectra among three oils, especially at selected frequency regions marked with (a), (b), and (c), were further optimized for simultaneous quantitative analysis of red fruit oil and sesame oil.

Quantitative Analysis

For simultaneous quantitative analysis of red fruit oil and sesame oil, two multivariate calibrations namely partial least square (PLS) regression and principle component regression (PCR) were used. PLS calibration is relied on its ability to exploit spectral information from wide range spectral frequencies and to correlate spectral changes as a function of the changes in the level of analytes of interest (red fruit oil and sesame oil) (Syahariza *et al.*, 2005). PLS calibration model was developed based on the calibration standard that included the different weighted amounts of analytes. Meanwhile, PCR exploit the combination of linear regression and principal component regression (Che Man *et al.*, 2010).

Based on the differences in FTIR spectra of red fruit oil and sesame oil marked with (a), (b), and (c), for frequency regions namely the whole spectra region (4000 – 650 cm^{-1}), 1780-1680 cm^{-1} , 1200-1000 cm^{-1} and the combined frequency regions of 1780-1680 and 1200-1000 cm^{-1} were investigated. Besides,

Table 2. The performance of multivariate calibration for analysis of sesame oil

Frequency region (cm ⁻¹)	Multivariate calibration	Spectra	Calibration			Validation		
			Equation	R ²	RMSEC	Equation	R ²	RMSEP
4000-650	PLS	normal	$y = 0.9869x + 0.5450$	0.9907	1.7161	$y = 0.9794x + 1.1724$	0.9927	1.4430
	PLS	1 st der	$y = 0.9899x + 0.4688$	0.9882	1.8234	$y = 0.9784x + 1.6006$	0.9906	3.5507
	PLS	2 nd der	$y = 0.9891x + 0.5117$	0.9871	1.8654	$y = 0.9774x + 1.7147$	0.9898	3.9335
	PCR	normal	$y = 0.9865x + 0.5582$	0.9906	1.7221	$y = 0.9795x + 1.2080$	0.9926	1.6495
	PCR	1 st der	$y = 0.9899x + 0.4689$	0.9882	1.8246	$y = 0.9784x + 1.6067$	0.9906	3.5741
	PCR	2 nd der	$y = 0.9890x + 0.5142$	0.9870	1.8680	$y = 0.9807x + 1.5836$	0.9898	4.0051
1780-1680	PLS	normal	$y = 0.9911x + 0.4066$	0.9897	1.7622	$y = 0.9826x + 1.3744$	0.9917	3.3274
	PLS	1 st der	$y = 0.9893x + 0.5049$	0.9868	1.8748	$y = 0.9784x + 1.7372$	0.9895	4.2766
	PLS	2 nd der	$y = 0.9883x + 0.5571$	0.9853	1.9248	$y = 0.9771x + 1.8684$	0.9885	4.6981
	PCR	normal	$y = 0.9911x + 0.4087$	0.9897	1.7622	$y = 0.9864x + 1.2170$	0.9917	3.3720
	PCR	1 st der	$y = 0.9893x + 0.5064$	0.9868	1.8762	$y = 0.9783x + 1.7417$	0.9895	4.3048
	PCR	2 nd der	$y = 0.9883x + 0.5558$	0.9852	1.9282	$y = 0.9766x + 1.9056$	0.9884	4.7881
1200-1000	PLS	normal	$y = 0.9921x + 0.3326$	0.9916	1.6767	$y = 0.9789x + 0.8576$	0.9936	0.4104
	PLS	1 st der	$y = 0.9910x + 0.4055$	0.9894	1.7766	$y = 0.9760x + 1.6034$	0.9916	2.9859
	PLS	2 nd der	$y = 0.9909x + 0.4124$	0.9893	1.7801	$y = 0.9749x + 1.7294$	0.9916	3.4089
	PCR	normal	$y = 0.9920x + 0.3425$	0.9916	1.6760	$y = 0.9789x + 0.8578$	0.9936	0.4096
	PCR	1 st der	$y = 0.9912x + 0.4020$	0.9894	1.7765	$y = 0.9760x + 1.6037$	0.9915	2.9861
	PCR	2 nd der	$y = 0.9908x + 0.4181$	0.9893	1.7803	$y = 0.9749x + 1.7299$	0.9916	3.4095
	1780-1680 and 1200-1000	PLS	normal	$y = 0.9918x + 0.3651$	0.9908	1.7121	$y = 0.9818x + 1.1838$	0.9927
PLS		1 st der	$y = 0.9895x + 0.4939$	0.9871	1.8643	$y = 0.9782x + 1.7233$	0.9898	4.1833
PLS		2 nd der	$y = 0.9885x + 0.5461$	0.9856	1.9146	$y = 0.9770x + 1.8575$	0.9888	4.6264
PCR		normal	$y = 0.9921x + 0.3505$	0.9908	1.7150	$y = 0.9818x + 1.1848$	0.9927	2.0871
PCR		1 st der	$y = 0.9895x + 0.4986$	0.9871	1.8651	$y = 0.9782x + 1.7273$	0.9897	4.1986
PCR		2 nd der	$y = 0.9884x + 0.5491$	0.9856	1.9169	$y = 0.9767x + 1.8837$	0.9887	4.6932

Table 3. The performance of multivariate calibration for analysis of red fruit oil

Frequency region (cm ⁻¹)	Multivariate calibration	Spectra	Calibration			Validation		
			Equation	R ²	RMSEC	Equation	R ²	RMSEP
4000-650	PLS	normal	$y = 0.9869x + 0.7650$	0.9907	1.7161	$y = 0.9794x + 0.8874$	0.9927	1.4427
	PLS	1 st der	$y = 0.9899x + 0.5396$	0.9882	1.8234	$y = 0.9784x + 0.5579$	0.9906	3.5507
	PLS	2 nd der	$y = 0.9891x + 0.5793$	0.9871	1.8654	$y = 0.9774x + 0.5441$	0.9898	3.9335
	PCR	normal	$y = 0.9865x + 0.7937$	0.9906	1.7221	$y = 0.9795x + 0.8469$	0.9926	1.5644
	PCR	1 st der	$y = 0.9899x + 0.5409$	0.9882	1.8246	$y = 0.9784x + 0.5639$	0.9906	3.6214
	PCR	2 nd der	$y = 0.9890x + 0.5828$	0.9870	1.8680	$y = 0.9807x + 0.3485$	0.9898	4.0051
1780-1680	PLS	normal	$y = 0.9911x + 0.4793$	0.9897	1.7622	$y = 0.9826x + 0.3639$	0.9917	3.3274
	PLS	1 st der	$y = 0.9893x + 0.5628$	0.9868	1.8748	$y = 0.9784x + 0.4260$	0.9895	4.2879
	PLS	2 nd der	$y = 0.9883x + 0.6127$	0.9853	1.9248	$y = 0.9771x + 0.4225$	0.9885	4.6981
	PCR	normal	$y = 0.9911x + 0.4773$	0.9897	1.7622	$y = 0.9864x + 0.1463$	0.9917	3.3720
	PCR	1 st der	$y = 0.9893x + 0.5647$	0.9868	1.8762	$y = 0.9783x + 0.4247$	0.9895	4.3048
	PCR	2 nd der	$y = 0.9883x + 0.6150$	0.9852	1.9282	$y = 0.9770x + 0.4209$	0.9885	4.7249
1200-1000	PLS	normal	$y = 0.9921x + 0.4605$	0.9916	1.6767	$y = 0.9789x + 1.2551$	0.9936	0.4104
	PLS	1 st der	$y = 0.9910x + 0.4934$	0.9894	1.7766	$y = 0.9760x + 0.7949$	0.9916	2.9859
	PLS	2 nd der	$y = 0.9909x + 0.4973$	0.9893	1.7801	$y = 0.9749x + 0.7795$	0.9916	3.4089
	PCR	normal	$y = 0.9920x + 0.4624$	0.9916	1.6760	$y = 0.9789x + 1.2550$	0.9936	0.4096
	PCR	1 st der	$y = 0.9912x + 0.4820$	0.9894	1.7765	$y = 0.9760x + 0.7953$	0.9915	2.9861
	PCR	2 nd der	$y = 0.9908x + 0.4978$	0.9893	1.7803	$y = 0.9749x + 0.7800$	0.9916	3.4095
	1780-1680 and 1200-1000	PLS	normal	$y = 0.9918x + 0.4502$	0.9908	1.7121	$y = 0.9818x + 0.6372$	0.9927
PLS		1 st der	$y = 0.9895x + 0.5533$	0.9871	1.8643	$y = 0.9780x + 0.4587$	0.9897	4.2246
PLS		2 nd der	$y = 0.9885x + 0.6025$	0.9856	1.9146	$y = 0.9770x + 0.4384$	0.9888	4.6264
PCR		normal	$y = 0.9921x + 0.4375$	0.9908	1.7150	$y = 0.9818x + 0.6367$	0.9927	2.0871
PCR		1 st der	$y = 0.9895x + 0.5537$	0.9871	1.8651	$y = 0.9782x + 0.4504$	0.9897	4.1986
PCR		2 nd der	$y = 0.9884x + 0.6101$	0.9856	1.9169	$y = 0.9770x + 0.4368$	0.9887	4.6513

the optimization was also performed on FTIR spectra either in normal spectra or in its derivative spectra (first and second derivatives). The selection of frequency regions and FTIR spectral treatment was based on the highest values of R² and the lowest values of RMSEC obtained during developing PLS calibration model.

Table 2 and 3 showed the performance of PLS and PCR calibrations for determination of sesame oil and red fruit oil simultaneously in terms of R² and root mean square error of calibration (RMSEC) values. It is known that PLS using normal spectra and at frequency region of 1200-1000 cm⁻¹ was preferred for quantification of both oils due to its ability to provide the highest value of R² and the lowest value of RMSEC.

Figure 2 exhibited the partial least square regression model for the relationship between actual and FTIR predicted values of sesame oil 1200-1000 cm⁻¹ showing the close relationship. Furthermore, this calibration model was further used for computing the levels of sesame oil in prediction samples. Figure 3 revealed the prediction model for the relationship between actual value of sesame oil and predicted value. Figure 4 and 5 exhibited the regression linear obtained for the relationship between actual value of red fruit oil (x-axis) and FTIR predicted value of red fruit oil (y-axis) in calibration and prediction samples, respectively.

Conclusion

Based on these results, it can be deduced that FTIR spectroscopy in combination with multivariate calibrations is promising technique for simultaneous determination of red fruit oil and sesame oil. FTIR spectra using normal mode at frequency regions of 1200 – 1000 cm^{-1} was suitable for such determination.

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