

Analysis of corn and soybean oils in red fruit oil using FTIR spectroscopy in combination with partial least square

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Abstract

The study highlighted the application of Fourier transform infrared (FTIR) spectroscopy as a non destructive and fast technique in the determination of red fruit oil (RFO) authenticity. RFO was made in binary mixture with corn oil (CO) and soybean oil (SO). Multivariate calibration method, partial least square (PLS), was used to construct the calibration model that correlate between actual and predicted values of RFO contents in the mixture at the FTIR spectral frequencies of 1800 – 1600 and 1200 – 800 cm⁻¹. Attenuated total reflectance (ATR) measurements were made on pure RFO and that adulterated with varying concentrations of CO and SO (1– 50% v/v). PLS at these frequencies showed the best calibration model, in terms of the highest coefficient of determination (R²) and the lowest of root mean square error of calibration (RMSEC), with R² of 0.999 and RMSEC of 0.987% (v/v), respectively for analysis of CO in the mixture with RFO. Meanwhile, the R² and RMSEC values obtained for the quantification of SO were of 0.997 and 1.195%. In general, FTIR spectroscopy serves as a suitable technique for determination of CO and SO in RFO for authenticity studies.

Keywords

FTIR spectroscopy
Authentication
Red fruit oil
Binary mixture
Partial least square

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Introduction

Red Fruit (*Pandanus conoideus* Lam) is typical plant or endemic which grow at Papua Island. Red fruit is 55 cm - 100 cm long, 10-15 cm diameter, and 2-3 kg weight. Fruit shape is oval with bright maroon red color and its bud covered with fruit leaves (Bourke, 2005). Its oil known as red fruit oil (RFO) can be taken into account as functional food oil. It is not only served as a diet but also as preventive agents of various diseases. RFO has been reported to have antioxidant activities in vitro (Rohman *et al.*, 2010a). Research related to anticancer of red fruit has been studied by Munim *et al.* (2006). Red fruit contains a fairly complete of essential vitamins and minerals. In addition, it also contains beta-carotene and tocopherol in high doses as well as oleic and linoleic acids (Budi and Paimin, 2004).

Authentication of red fruit oil (RFO) becomes very important since the price of RFO is 10-15 times higher than that of common vegetable oils (Rohman *et al.*, 2011a). The oil can be easily faked with other cheaper oils such as corn oil, palm oil, sesame oil, and others. Determination of the authenticity of high price oils is an interesting issue not only for oil producers but also for consumers, as it associated with the health of consumers, and in many ways, related to the prohibition of certain religions like adulteration

of vegetable oil with lard (Rohman and Che Man, 2008; Rohman and Che Man, 2012). However, the determination of oil adulterants traditionally requires time and a long process, typically using chromatographic such as gas chromatography-combustion- isotope ratio mass spectrometer (GC-C-IRMS) technique (Schipilliti *et al.*, 2011) and wet chemical methods (Rohman and Che Man, 2009a). Therefore, it needed a method to detect any adulteration of oil quickly, simply and reliably.

Fourier transform infrared (FTIR) spectroscopy has been widely used in food research and has become a powerful analytical tool in the study of edible fats and oils, especially for qualitative identification of specific components in food (Che Man *et al.*, 2010). FTIR spectroscopy-based methods are fast, sensitive, non destructive, with minimum sample preparation requirement (Rohman dan Che Man, 2009a). IR spectroscopy is known as a fingerprint technique since no two compounds have a number of peaks or absorbance with the same intensity (Rohman, 2012). Combined with computerized chemometrics and software, FTIR spectroscopy can provide qualitative and quantitative information (Rohman *et al.*, 2010b). For this reason, this study will combine both methods (FTIR spectroscopy and chemometrics) to detect and quantify the existed adulteration on red fruit oil.

Chemometrics can be described as the application

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of mathematical and statistical methods to improve chemical measurement processes and to extract more useful chemical information from chemical and physical measurement data. Multivariate calibration is one of the useful chemometrics techniques for analysis of complex mixtures, as it enables the rapid and simultaneous determination of each component in the mixture without time-consuming separation and with minimum sample preparation (Maggio *et al.*, 2009). FTIR spectroscopy combined with multivariate calibration of partial least square (PLS) has been reported for the analysis of certain oils in the mixture with others for authentication issues such as soybean, peanut, corn, and sesame oils in olive oil, hazelnut oil in refined olive oil, sunflower, corn, soybean and hazelnut oils in extra virgin olive oil, and lard in cod-liver oil (Rohman and Che Man, 2009a), lard in vegetable oils (Rohman *et al.*, 2011b), palm oil in virgin coconut oil (Rohman and Che Man, 2009b) as well as canola oil in virgin coconut oil (Che Man and Rohman, 2013).

However, little information is available regarding the analysis of corn oil (CO) and soybean oil (SO) as adulterants in RFO using FTIR spectroscopy. In this research, we have developed FTIR spectroscopic technique for monitoring RFO adulteration with corn and soybean oils using chemometrics of PLS for authentication of RFO.

Materials and Methods

Materials

Red Fruit was taken from Papua, Indonesia. Botanical identification was performed by Department of Pharmaceutical Biology, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, Indonesia. Corn Oil (CO) and Soybean Oil (SO) were purchased from the local market in Jogjakarta, Indonesia.

Sample preparation

For quantitative analysis using partial least square (PLS) calibration, a set of 20 samples containing red fruit oil (RFO) and SO as well as RFO with CO was mixed together in accurately weighted proportions of 1 – 50% v/v, and shaken vigorously to ensure the total homogenization. The spectral regions where the variations were observed were chosen for developing PLS model for analysis of adulterants (CO and SO) in RFO. PLS analysis relates FTIR absorbance of adulterant (CO and SO) with the percentages of adulterant. The predictability of the models was tested by computing the root mean square error of calibration (RMSEC) for the calibration data set, and the root mean square error of prediction (RMSEP)

for the validation data set:

$$\text{RMSEC} = \sqrt{\frac{\sum_{i=1}^n (\text{actual} - \text{calculated})^2}{N - f - 1}}$$

The term actual refers to the known concentration of selected data. Meanwhile, the calculated refers to a value computed by the model using FTIR spectral data. N is the number of samples used in the calibration sets, and f is the number of factors used in the calibration model.

To evaluate the goodness of fit in the validation, root mean square error of prediction (RMSEP) is calculated using the following equation:

$$\text{RMSEP} = \sqrt{\frac{\sum_{i=1}^m (\text{actual} - \text{calculated})^2}{M - 1}}$$

where M is the number of samples used in the prediction sets (Rohman *et al.*, 2011).

Measurement of FTIR spectra

FTIR spectrophotometer was used to analyze all samples, either for calibration or validation models. 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). FTIR spectrometer equipped with a deuterated triglycine sulphate (DTGS) as a detector and a KBr/germanium as beam splitter, interfaced to computer operating under Windows-based, and connected to software of the HORIZON MBTM, was used during FTIR spectra acquisition. A few drops of each sample were positioned in contact with attenuated total reflectance (ATR) on a multi-bounce plate of crystal at controlled ambient temperature (25°C). After every scan, a new reference air background spectrum was taken. The ATR plate was carefully cleaned by scrubbing with hexane twice followed by acetone and dried with soft tissue before filling in with the next sample. ATR cleanliness was verified by collecting a background spectrum and compare to the previous one. For the sake quantitative analysis, these FTIR spectra were recorded as absorbance values at each data point in triplicate (Rohman *et al.*, 2010).

Statistical Analysis

Data analysis using PLS was performed using the HORIZON MBTM FTIR software. Frequency regions for PLS were automatically selected by the software and were confirmed by investigating peaks where variations were observed. The values of root mean square error of calibration (RMSEC) and coefficient of determination (R^2) were used as the

Table 1. Functional groups and mode of vibration from FTIR spectra of the evaluated oils

Peak position on FTIR spectra (cm ⁻¹)	Assignment of bonds	Mode of vibration
3005	=C-H (<i>cis</i>)	stretching
2953	-C-H (CH ₃)	Stretching asymmetric
2922	-C-H (CH ₂)	Stretching asymmetric
2853	-C-H (CH ₂)	Stretching symmetric
1746	-C=O (ester)	stretching
1654	-C=C- (<i>cis</i>)	stretching
1463	-C-H (CH ₂)	Bending (scissoring)
1417	=C-H (<i>cis</i>)	Bending (rocking)
1377	-C-H (CH ₃)	Bending symmetric
1160	-C-O	Stretching
	-CH ₂ -	Bending
1118, 1098, 1030	C-O	Stretching
962	<i>trans</i> -CH=CH-	bending out of plane
854	=CH ₂	wagging
757	-C-H	bending out of plane
722	<i>cis</i> -CH=CH-	bending out of plane

(Lerma-Garcia *et al.*, 2010)

validity criteria for the calibration.

Results and Discussion

FTIR spectral analysis

Figure 1 shows the typical FTIR spectra of the RFO, CO and SO used in this study. The analytical evaluation of the RFO spectrum in term of functional groups corresponding for absorption of certain frequencies/wavenumbers is given in Table 1. As shown in Figure 1, it is difficult to differentiate among these oils by just visual examination of their whole spectra. However, if one examines the spectra scrutiny, they reveal some differences in their FTIR spectra which can be observed in region around 1700-1780 cm⁻¹ and at fingerprint region (1500-650 cm⁻¹). There is no band at 1712 cm⁻¹ for CO and SO, and the otherwise was observed for RFO (attributed from the stretching vibration of C=O). Furthermore, at spectral region of 1000-1150 cm⁻¹, RFO has two peaks, meanwhile CO and SO reveal one peak. At frequency region of 910-960 cm⁻¹, these oils also revealed different peak intensities due to the difference of fatty acids composition. These differences can be used as guidance for the optimization detection and of quantification of CO and SO as adulterant in RFO.

Quantification of corn oil and soybean oil in red fruit oil

Quantification of corn oil contents in the RFO-adulterated samples was performed using partial least square (PLS) algorithms. The PLS models were constructed based on inverse calibration modeling, commonly used in the estimation of concentration in multi-component mixtures (Rohman *et al.*, 2011b). The samples of all the adulterated and pure red fruit oils were randomly divided into a calibration and a validation sets. The calibration set considered of 41 samples, and the validation set used of 20 samples. The division into both sets was done in order to obtain the similar mean values and standard deviation, so

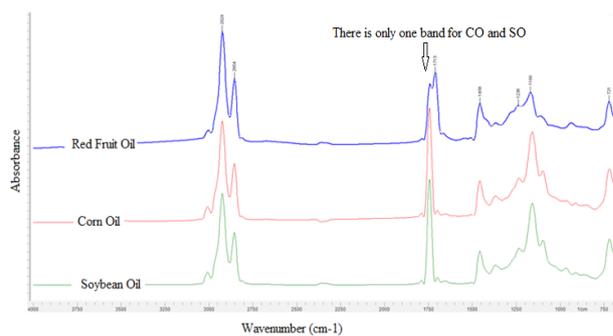


Figure 1. FTIR Spectra of Red Fruit Oil (RFO), Corn Oil (CO), and Soybean Oil (SO) at wavenumber 4000-650cm⁻¹

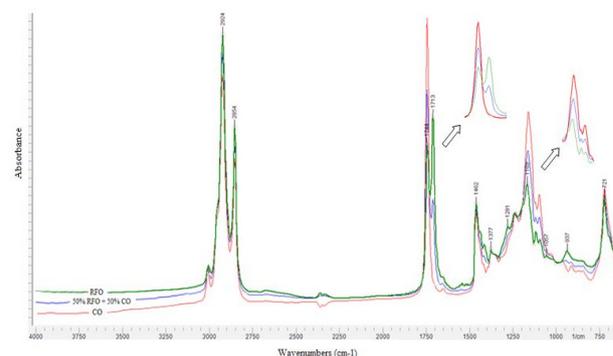


Figure 2. FTIR spectra of Red Fruit Oil (RFO) and Corn Oil (CO) at wavenumber of 4,000-650 cm⁻¹

both sets of samples spanned the full range of RFO contents (Rohman *et al.*, 2011b).

Analysis of corn oil in RFO

Figure 2 revealed FTIR spectra of RFO and corn oil which can be differentiated at frequency regions of 1700-1780 cm⁻¹ and at fingerprint region of 1500-650 cm⁻¹. Using the optimization process, the combined frequency region of 1600-1800 cm⁻¹ and 800-1200 cm⁻¹ were selected for quantification of CO in RFO due to its ability to obtain the lowest values of RMSEC and the highest values of R². In the PLS calibration models, the evaluation of the method linearity was carried out in order to show the proportional relationship between absorbance versus analyte concentrations.

Figure 3 shows the relationship between actual and calculated values of corn oil in RFO indicating the close correlation with coefficient of determination (R²) of 0.998. Furthermore, the value of RMSEC was used to evaluate the error in calibration model. As shown in Table 2, the RMSEC value of CO in RFO obtained is 0.987% (v/v) for normal FTIR spectra, 0.994% (v/v) for second derivative spectral, and 1.205% (v/v) for first derivative spectral. The low value of RMSEC indicates the good performance of PLS model. Based on this result, it can be stated that the level of CO as low as of 1% (v/v) in RFO could be detected.

Table 2. The performance of PLS for determination of corn and soybean oils in red fruit oil

Spectral treatments	CO			SO		
	R ²	RMSEC	RMSEP	R ²	RMSEC	RMSEP
Normal	0.999	0.987	0.985	0.997	1.195	1.063
1 st derivative	0.996	1.205	1.435	0.996	1.575	1.396
2 nd derivative	0.998	0.994	0.988	0.997	1.369	1.217

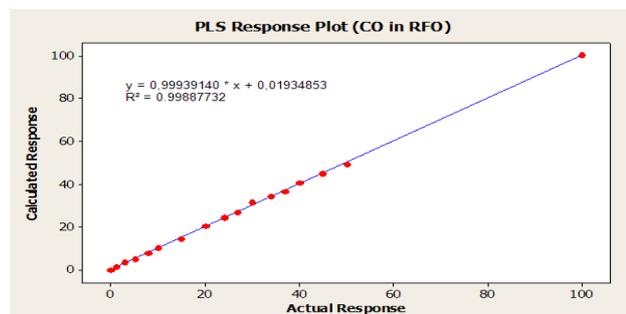


Figure 3. PLS calibration model for the relationship between actual and estimated concentrations of corn oil in RFO at normal spectra

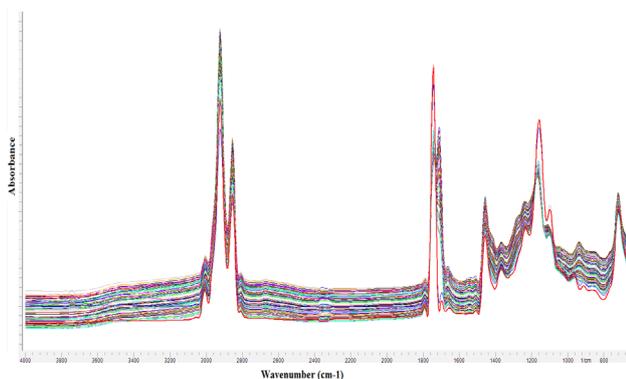


Figure 4. Overlay spectra of soybean oil mixed into red fruit oil at concentration range of 1.0–50.0% (v/v)

In order to validate the developed model, a set series of independent samples were used. The developed calibration model was exploited for quantification of validation samples, and a parameter called with root mean square error of prediction/validation (RMSEP) was computed (Gurdeniz and Ozen, 2009). The RMSEP value of CO in RFO obtained is 1.195% (v/v) for normal spectral, 1.575% (v/v) for second derivative spectra, and 1.369% (v/v) for first derivative spectra, respectively. The low value of RMSEP and the high R² value demonstrate high predictive ability of calibration model to validation samples. This result shows that FTIR spectroscopy coupled with PLS using the normal spectra at frequencies of 1600-1800 cm⁻¹ and 800-1200 cm⁻¹ is an accurate technique for quantification of CO as adulterant in RFO.

Analysis of soybean oil in RFO

Figure 4 exhibited the FTIR spectra of RFO in mixture with SO in the range of 0 – 50% SO in RFO

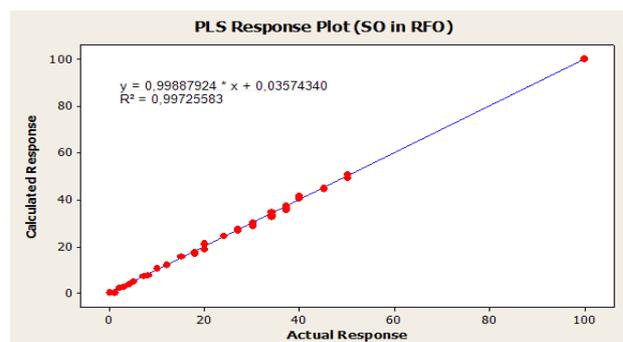


Figure 5. PLS calibration model for the relationship between actual and estimated concentrations of soybean oil in RFO at normal spectra.

at wavenumbers 4,000 – 650 cm⁻¹. The difference between RFO and SO spectrum can be seen in the frequency region of 1712 cm⁻¹ and 1098 cm⁻¹. These frequencies were employed for making the calibration and validation models using PLS calibration technique for determination of RFO adulterated with SO in the spectral range of 1600-1800 cm⁻¹ and 800-1200 cm⁻¹. The values of R², RMSEC, and RMSEP from the normal spectra, first and second derivatives are shown in table 2.

It can be shown that the normal spectra in combination with PLS calibration technique at two frequencies of 1600-1800 cm⁻¹ and 800-1200 cm⁻¹ is the better model, in terms of the highest R² (0.997), the lowest values of RMSEC (1.195 % v/v) for the relationship between the actual value and the predicted SO contents with the equation of $y = 0.999x + 0.036$ (Figure 5). The validation/prediction model showed that the RMSEP value obtained was 1.063 % (v/v). The R² 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 in independent samples. The high value of R² and the low value of RMSEP indicated that the PLS calibration model was suitable for determining SO in the mixture of RFO. Besides, based on RMSEC values, it can be assumed that the detection limit of SO in RFO as determined using FTIR spectroscopy is as low as of 1% v/v.

Conclusion

It can be concluded that FTIR spectroscopy combined with multivariate calibrations can be used to monitor the adulteration of RFO with corn and soybean oils. PLS calibrations can be successfully used to quantify the level of adulterants (corn oil and soybean oil) contents at the combined frequencies of 1600-1800 cm⁻¹ and 800-1200 cm⁻¹.

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