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Analysis of candlenut oil as oil adulterant in three functional oils of soybean oil, sunflower oil and grapeseed oil in quaternary mixture systems using FTIR spectroscopy and chemometrics

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Abstract

Soybean oil (SBO), sunflower oil (SFO) and grapeseed oil (GPO) contain high levels of unsaturated fats that are good for health and have proximity to candlenut oil. Candlenut oil (CNO) has a lower price and easier to get oil from that seeds than other seed oils, so it is used as adulteration for gains. Therefore, authentication is required to ensure the purity of oils by proper analysis. This research was aimed to highlight the FTIR spectroscopy application with multivariate calibration is a potential analysis for scanning the quaternary mixture of CNO, SBO, SFO and GPO. CNO quantification was performed using multivariate calibrations of principle component (PCR) regression and partial least (PLS) square to predict the model from the optimization FTIR spectra regions. The highest R^2 and the lowest values of root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) were used as the basis for selection of multivariate calibrations created using several wavenumbers region of FTIR spectra. Wavenumbers regions of 4000-650 cm^{-1} from the second derivative FTIR-ATR spectra using PLS was used for quantitative analysis of CNO in quaternary mixture with SBO, SFO and GPO with R^2 calibration = 0.9942 and 0.0239% for RMSEC value and 0.0495%. So, it can be concluded the use of FTIR spectra combination with PLS is accurate to detect quaternary mixtures of CNO, SBO, SFO and GPO with the highest R^2 values and the lowest RMSEC and RMSEP values.

1. Introduction

Candlenut (*Aleurites mollucana* (L.) willd.) oil is a plant that is able to produce oil up to 3200 kg/ha and it is able to produce 30-50% oil in every kilogram (Riyanta *et al.*, 2020a). The size of candlenut seeds is larger than soybean seeds, sunflower seeds and even grapeseed make candlenut seeds have higher oil potential. However, candlenut oil is more easily produced than other oils and has the same proximity characteristics as soybean oil, sunflower seed oil and grape seed oil. Extraction methods that have been used like pressing, maceration, soxhletation, sonication and using supercritical CO_2 (Siddique *et al.*, 2015; Aryati *et al.*, 2020; Riyanta *et al.*, 2020b)

Fourier transform infrared (FTIR) in combination with chemometrics of classification such as discriminant analysis and multivariate calibration of partial least square (PLS) and principle component regression has been used for authentication analysis of raw milk (Coitinho *et al.*, 2017), motor oil adulteration (Bassbasi *et al.*, 2013), pumpkin seed oil (Irnawati *et al.*, 2019), olive oil (Nurwahidah *et al.*, 2019), sesame oil (Nurrulhidayah *et al.*, 2014), beef meatball (Rahayu *et al.*, 2018), and fish patin oil (Putri *et al.*, 2019). However, using literature review, there are no reports regarding the analysis candlenut oil (CNO) in the quaternary mixture from other oils using FTIR spectroscopy. However, it is often found that oil products are mixed with other oils to reduce production costs. Therefore, in this study, FTIR spectroscopy combined with chemometrics of multivariate calibrations was performed to analyze of candlenut oil in quaternary oil mixture.

2. Materials and methods

2.1 Materials

The candlenuts (*Aleurites mollucana* (L) Willd) were

collected from Bumiayu, Brebes, Central Java in February 2019. The candlenuts were collected from seed that has fallen from tree and seeds were opened from the shell by gouging out. The candlenut oils were extracted using apparatus oil press according to Riyanta *et al.* (2020a). The candlenut oil was collected in a brown bottle and stored in the freezer before used for analysis. For soybean oil, sunflower oil, and grapeseed oil were purchased from local markets in Yogyakarta, Indonesia.

2.2 Samples preparation for calibration and validation

Candlenut oils in a quaternary mixture of soybean oil, sunflower oil and grapeseed oil were prepared at concentrations in the range of 0-100.0% v/v. Samples were prepared randomly by Microsoft Office Excel (Inc. USA) and shown in Table 1. Then analyzed using FTIR spectrophotometer.

Table 1. The composition of candlenut oil (CNO) in quaternary mixtures with soybean oil (SBO), sunflower oil (SFO) and grapeseed oil (GPO)

No	Percentage (%) composition of oils			
	SBO	SFO	GSO	CNO
1	29.00	26.00	36.00	9.00
2	13.71	8.13	8.20	69.97
3	22.08	5.78	37.05	35.05
4	15.31	14.66	7.36	62.66
5	27.08	35.07	30.24	7.62
6	2.47	41.70	45.24	10.59
7	17.76	10.80	3.03	68.40
8	25.95	8.54	26.73	38.78
9	8.08	23.45	31.40	37.07
10	43.58	10.51	35.78	10.14
11	30.05	11.64	24.98	33.34
12	26.66	22.66	48.12	2.56
13	35.45	25.48	18.23	20.85
14	11.51	14.05	13.97	60.47
15	6.42	9.55	44.28	39.75
16	6.48	6.48	35.66	51.38
17	8.22	43.26	29.37	19.15
18	16.75	10.16	47.81	25.28
19	6.25	29.48	12.05	52.22
20	13.00	49.00	37.50	0.00
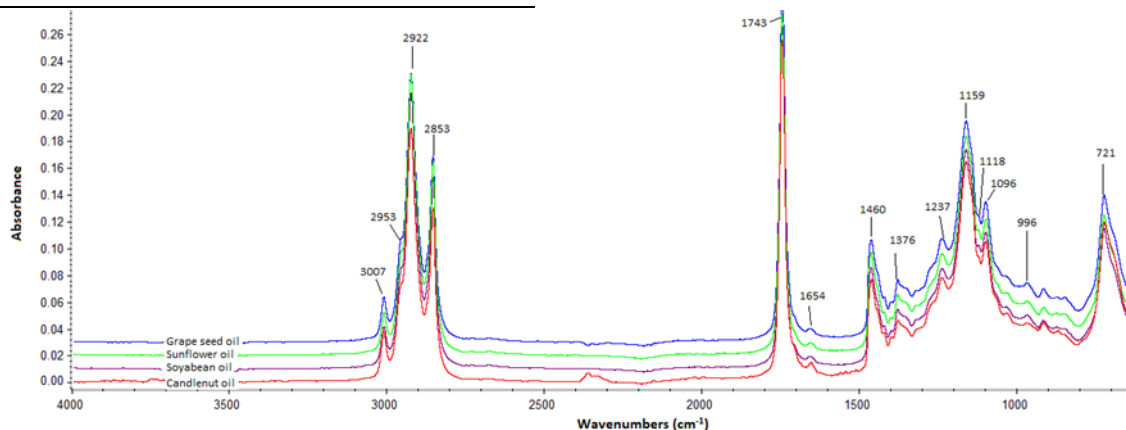
2.3 FTIR measurement

Oil samples were scanning with FTIR spectrophotometer (Scientific Nicolet, Madison, WI) with Omnic software for FTIR spectra processing. Scans 4000-650 cm^{-1} of wavenumber with thirty-two scanning and for resolution applied 8 cm^{-1} of Horizontal Attenuated Total Reflectance at wavenumbers. As background were used air for all FTIR spectra and taken every scan for new reference scanning.

2.4 Chemometrics analysis

TQ Analyst software (version 9) by Thermo Fisher Scientific, Inc was used for chemometrics analysis multivariate calibration and discriminant analysis. Partial least square (PLS) regression and principle (PCR) component regression was used for quantification of oils.

3. Results and discussion

FTIR spectroscopy is effectively able to analyze mixed sample like the quaternary mixture of oils. Quantitative analysis can be done by processing FTIR spectra in the form of signals from analytic peak and shoulders to be used as dependent variables using a calibration model. For this research, attenuated total reflectance (ATR) was used as a sampling technique, in where the samples (without any addition of solvent) were directly placed into ATR crystal. The combination of FTIR and multivariate analysis was successfully modelled for the oil adulteration mixture (López-Díez *et al.*, 2003). Principle component (PCR) regression and partial least square (PLS) regression in combination with FTIR-ATR were more powerful technique for quantitative analysis (Rohman and Man, 2010; Irnawati *et al.*, 2020). Figure 1 shows the FTIR-ATR spectra in the regions of wavenumber from 4000-650 cm^{-1} for oil mixture spectra of CNO, SBO, SFO, and GPO.

Wavenumber 3010-2800 cm^{-1} at peak 3007 was stretching vibration of cis C=CH, while the peak at 2953

Figure 1. FTIR spectra of candlenut oil, soybean oil, sunflower oil and grape seed oil at mid-infrared region (4000-650 cm-1) measured using attenuated total reflectance mode

cm^{-1} from asymmetric stretching vibration of methyl (-CH_3) group. Asymmetric and symmetric stretching vibrations of methylene (-CH2) showed by the peaks at 2922 and 2853 cm^{-1}. The wavenumber 1800-1600 cm^{-1} showed the stretching vibration from carbonyl (C=O) was observed at 1744 cm^{-1}, while the peak at 1654 is from C=C stretching vibration. The wavenumber 1500-650 were fingerprinting area showed with bending vibrations of methylene and methyl were observed at wavenumbers of 1460 and 1376 cm^{-1}, respectively. The peaks at regions of 1237, 1160, 1118, 1098 were from C-O vibrations. While, peaks at 996 and 850 were due to bending out of plane vibrations of – HC=CH– (trans) and –HC=CH– (cis), respectively (Inarwarti *et al.*, 2020).

The analytical method of FTIR spectra for fingerprint can be used to detect for adulteration and these methods most ideal for detection and quantification of adulterants in mixed samples. Detection of CNO as an adulterant in quaternary mixture with soybean oil (SBO), sunflower oil (SFO), and grapeseed oil (GPO) was performed using optimized spectra regions of FTIR combined with multivariate calibrations. Principle component regression (PCR) and partial least square (PLS) were used as predictor models of principle components of oils mixture (Khudzaifi *et al.*, 2020). Prediction models between actual values of CNO and FTIR predicted values were optimized by PLS and PCR from FTIR spectra. The highest coefficient of determination (R^2) and the lowest root mean square of calibration (RMSEC) and root mean square error of prediction (RMSEP) were used as parameters to be decided as the FTIR spectral area for multivariate calibration.

Table 2 tabulates the prediction of CNO levels as an adulterant in with SBO, SFO and GPO by FTIR spectroscopy using different wavenumbers and spectral treatments combined with the multivariate calibration of PLS and PCR. Wavenumbers of 4000-650 cm^{-1} using second derivative FTIR spectra combined with PLS was selected for the quantification of CNO in SBO, SFO and GPO and able to provide the highest R^2 values (0.9942) either in calibration or prediction with low RMSEC value (0.0239) and RMSEP value (0.0495). High values of R^2 and low values of RMSEC and RMSEP were capable of developing models revealed the acceptable accuracy and precision. Figure 3A exhibits the linear relationship between actual values of CNO (x-axis) and FTIR predicted values of CNO (y-axis) using FTIR spectroscopy. From Figure 3B, the errors occurring during modelling occurred randomly (not systematic error) around zero difference. This indicated that the systematic errors did not exist and the developed model was reliable to predict CNO in mixture with SBO, SFO and GPO.

Discriminant analysis (DA) is known as the supervised pattern recognition commonly used for discrimination between pure CNO and CNO in the quaternary mixture (Riyanta *et al.,* 2020a). The variables used for quantitative analysis were also used for DA. The Mahalanobis distances using these absorbances were then calculated to create Cooman's plot. Figure 4 shows The Cooman's plot obtained during discriminant analysis for discrimination of pure *Candlenut* Oil (CNO) and CNO mixed with SBO, SFO, and GPO.

4. Conclusion

Combination of Fourier transform infrared (FTIR) spectroscopy and multivariate calibration of partial least square regression (PLS) and discriminant are capable for quantifying the levels of candlenut oil as an adulterant in

Table 2. The results of FTIR spectroscopy using different wavenumbers and spectral treatments coupled with multivariate calibration intended for prediction of soybean oil, sunflower oil, grapeseed oil levels adulterated with candlenut oil (CNO)

	Wavenumber (cm^{-1})	Spectra	Calculation		Validation	
			R^2	RMSEC	R^2	RMSEP
PCR	4000 – 650	Normal	0.9884	0.0337	0.9788	0.0475
		Derivative 1	0.9868	0.0361	0.9809	0.0442
		Derivative 2	**0.9878**	**0.0347**	**0.9722**	**0.0547**
	3050 - 2800	Normal	0.9885	0.0336	0.9752	0.0522
		Derivative 1	0.9877	0.0348	0.9815	0.0449
		Derivative 2	0.9809	0.0432	0.9776	0.0465
	1800 – 650	Normal	0.9891	0.0327	0.9808	0.0446
		Derivative 1	0.9899	0.0315	0.9853	0.0384
		Derivative 2	0.9884	0.0338	0.9758	0.052
	3000 – 2800 and 1600 - 650	Normal	0.9889	0.033	0.981	0.0431
		Derivative 1	0.9879	0.0345	0.9812	0.0434
		Derivative 2	0.9851	0.0382	0.9749	0.054

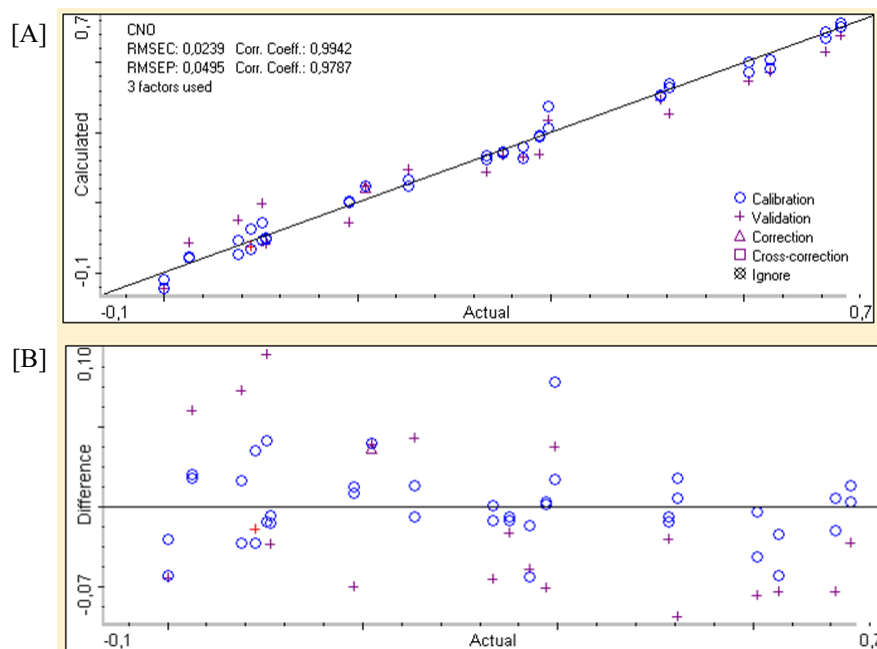**The selected variables were marked with bold.**

Figure 3. The relationship between actual values of Candlenut Oil (CNO) in x-axis and the predicted values of CNO in y-axis using FTIR spectroscopy [A] along with residual analysis [B]
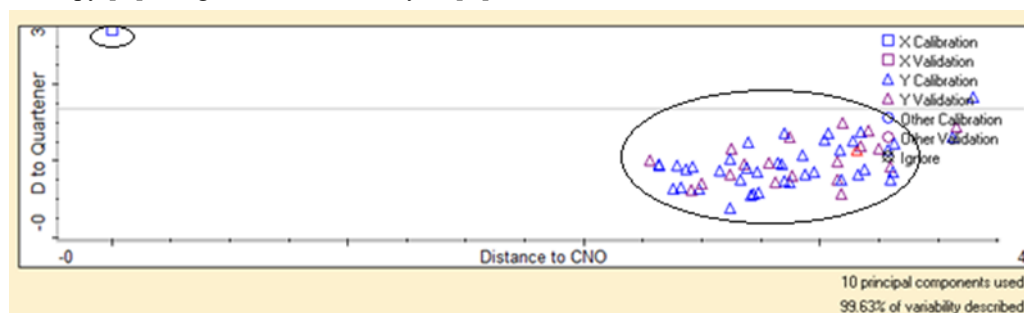

Figure 4. The Cooman's plot obtained during discriminant analysis for discrimination of pure Candlenut Oil (CNO) and CNO mixed with SBO, SFO, and GPO

soybean oil (SBO), sunflower oil (SFO) and grapeseed oil (GPO) in the quaternary mixture at optimized wavenumbers of 4000-650 cm^{-1}. The highest R^2 and lowest RMSEC and RMSEP model as acceptable accuracy and precision. Discriminant analysis can be used to classify CNO as an adulterant in soybean oil (SBO), sunflower oil (SFO), and grapeseed oil (GPO) mixtures.

Conflict of interest

We have no conflict of interest to declare.

Acknowledgement

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