

# The employment of FTIR spectroscopy and chemometrics for authentication of essential oil of *Curcuma mangga* from candle nut oil

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## Abstract

The adulteration of high price oil such as essential oil of *Curcuma mangga* Val. (EOCM) with lower price oil is common to get economical profit. This study was to investigate the authentication of EOCM toward candlenut oil (CNO) using FTIR spectroscopy combined with multivariate calibration and discriminant analysis. The selection of CNO as adulterant oil model was due to its close similarity to EOCM in terms of FTIR spectra. Besides, EOCM has similar color with CNO, therefore, CNO is potential adulterant toward EOCM. Two multivariate calibrations of partial least square regression (PLSR) and principle component regression (PCR) along with FTIR spectra (normal versus derivatization) were optimized to get prediction models for quantification. The results showed that the combination of PLSR and normal FTIR spectra at optimized wavenumbers of 1614-1068 cm<sup>-1</sup> was capable of predicting the levels of EOCM adulterated with CNO. Discriminant analysis was also success to differentiate the classification of EOCM and EOCM adulterated with CNO with accuracy levels of 100%. Using FTIR spectroscopy for oil authentication is rapid, simple without any chemicals, solvents, and sample preparation so that this technique is considered as a green analytical method.

## 1. Introduction

*Curcuma mangga* Valetton and Van Zijp., belonging to *Zingiberaceae* family, known as Temu mangga (Indonesia) possessing mango-like aroma, is used for flavouring steamed and baked fish due to its aromatic properties (Liu and Nair, 2019). Its rhizome contains curcuminoids and diterpenes which have several biological activities including anti-inflammatory (Kaewkroek *et al.*, 2010) and antioxidant effects through antiradical and reducing power evaluations (Indis and Kurniawan, 2016; Tamta *et al.*, 2016). This plant is reported to have wound healing effects after tissue damage (Sriroda and Tewtrakul, 2019). Besides, labdane diterpenes are also reported to inhibit lipid peroxidation, cyclooxygenase enzymes, and human tumour cell proliferation (Liu and Nair, 2011) and to treat gastric ulcer, chest pain, fever, and skin disease (Abas *et al.*, 2005).

The mango-like aroma in *C. mangga* originates from volatile components present in essential oils. Some chemical components have been identified from the

essential oil of *C. mangga* (EOCM). Wong *et al.* (1999) reported that EOCM contained 44 components in which monoterpene hydrocarbons were dominant, followed by  $\beta$ -myrcene, (E)- $\beta$ -ocimene,  $\beta$ -pinene and  $\alpha$ -pinene. Padalia *et al.* (2013) reported that EOCM was dominated by monoterpenoids comprising of  $\beta$ -myrcene,  $\beta$ -pinene, (E)- $\beta$ -ocimene. Some minor constituents like camphene, 1,8-cineole, limonene/ $\beta$ -phellandrene, curzerenone, caryophyllene oxide, borneol, as well as camphor, isoborneol, camphene, borneol and camphene hydrate have been reported (Tamta *et al.*, 2016). EOCM has been reported to show the strong antibacterial activities against *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, antifungal activity against *Candida albicans* and *Cryptococcus neoformans* (Kamazeri *et al.*, 2012) and antioxidant activities (Jena *et al.*, 2017).

Due to its activities, EOCM has a higher price value than other vegetable oils such as palm and corn oils, and it may be adulterated with other lower price oils. Because of such reasons, physicochemical analytical techniques which are reliable for adulteration analysis of

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EOCM are highly needed. Some methods have been proposed, developed and used for authentication of high price oils such as molecular spectroscopy (Lankmayr *et al.*, 2004), nuclear magnetic resonance spectroscopy (Dais and Hatzakis, 2013), differential scanning calorimetry (Van Wetten *et al.*, 2015), chromatographic techniques, namely high performance liquid chromatography (Flores *et al.*, 2006; Bajoub *et al.*, 2017), gas chromatography using flame ionization detector (Jafari *et al.*, 2009), gas chromatography in combination with atmospheric pressure chemical ionization-time of flight (García-Villalba *et al.*, 2011), and real-time polymerase chain reaction (Giménez *et al.*, 2010; Alonso-Rebollo *et al.*, 2017). These methods were time-consuming, needed sophisticated instruments and involved complex sample preparation, indeed, these methods were not preferred to be applied in routine monitoring systems. This condition has challenged some analytical chemists to develop a rapid and reliable technique, especially based on spectroscopic methods such as FTIR spectroscopy.

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 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 authentication of EOCM from other oils using FTIR spectroscopy. Therefore, in this study, FTIR spectroscopy combined with chemometrics of multivariate calibrations and classification of discriminant analysis was performed for authentication of EOCM from candlenut oil adulteration.

## 2. Materials and methods

### 2.1 Materials

The rhizomes of *Curcuma mangga* Val. were collected from Terong Village, Dlingo, Bantul, Yogyakarta on September 2018. The rhizomes were cleaned and thinly chopped into small pieces using a cutter. The chopped rhizomes were air-dried in a room for 3 days. The essential oils of rhizomes of *C. mangga* Val. (EOCM) oil was isolated using water steamed distillation according to Astuti *et al.* (2014). The distillation process was taken for 6 hours, and the obtained EOCM was collected in a small clean bottle and stored in a freezer until it was used for further authentication analysis. For the comparisons, garlic oil, extra virgin olive oil, corn oil, castor oil, citrus oil, caraway oil, soybean oil, coconut oil, candlenut oil,

cananga oil, sunflower oil, rose oil, palm oil, sesame oil and olive oil were purchased from several local markets and supermarkets around Yogyakarta, Indonesia.

### 2.2 Principal component analysis (PCA) of oils

The FTIR spectra of essential oils extracted from *C. mangga* Val. (EOCM) and the comparing oils were subjected to principal component analysis (PCA). Oil having similar score plots of first principle component (PC1) and second principle component (PC2) based on the absorbance values at whole mid infrared regions (4000-650  $\text{cm}^{-1}$ ) to that of EOCM was used as the oil adulterant (Che Man *et al.*, 2011).

### 2.3 Preparation of calibration and validation samples

In the quantification analysis, a series of calibration and validation or prediction samples with known concentrations of CNO and EOCM were prepared as in Irnawati *et al.* (2020). For preparing the calibration samples, twenty-one samples consisting of EOCM in the binary mixtures with CNO at concentration ranges of 0–100.0% v/v were made. For preparing the validation samples, a series of different samples were made in the concentration ranges covered by the calibration samples. All samples were analyzed using FTIR spectrophotometer.

### 2.4 Discriminant analysis (DA)

In the classification of genuine EOCM and that adulterated with CNO, a chemometric of supervised pattern recognition of discriminant analysis (DA) was used. The genuine EOCM and that adulterated with CNO were prepared in the concentration range of 0.5–50.0% (v/v). Pure EOCM was assigned as authentic, meanwhile, EOCM and that adulterated with CNO was assigned with “adulterated”. Both classes were classified and discriminated using DA on the basis of their FTIR spectra.

### 2.5 FTIR spectra acquisition

All FTIR spectra were acquired from FTIR spectrophotometer (Thermo Scientific Nicolet iS10, Madison, WI), controlled with the operating software of Omnic®. The measurements were performed in mid infrared region of 4000-650  $\text{cm}^{-1}$  with the resolution was 8  $\text{cm}^{-1}$  and the number of scanning of 32 using horizontal attenuated total reflectance (HATR) composed of ZnSe crystal. All FTIR spectra were corrected against the FTIR spectrum of air as the background. After every scan, a new reference air background spectrum was taken. To facilitate the quantitative analysis, these spectra were recorded as absorbance values at each data point in triplicate.

## 2.6 Chemometrics analysis

Chemometrics of classification using discriminant analysis and multivariate calibrations of partial least square regression was performed using TQ Analyst software version 9 (Thermo Fisher Scientific, Inc.). Principal component analysis of EOCM and other oils was carried out using software Minitab version 17 (Minitab Inc., USA).

## 3. Results and discussion

In this study, the essential oil of *Curcuma mangga* Val. (EOCM) was authenticated from other oils using FTIR spectroscopy in combination with several chemometrics techniques of unsupervised pattern recognition namely principal component analysis (PCA), supervised pattern recognition of discriminant analysis, and multivariate calibration of partial least square regression (PLSR). PCA was used to determine which oils having the closest similarity with EOCM based on the FTIR spectra. The output of PCA is known of score plot of first principle component analysis (PC1) and second principle component (PC2) in which samples having similar PC1 and PC2 are similar in nature depending the variables used (Rohman and Putri, 2019). Figure 1 revealed that the score plots of PC1 and PC2, EOCM has the closest similarity to that of candlenut oil (CNO), therefore CNO was selected as oil adulterant to EOCM.

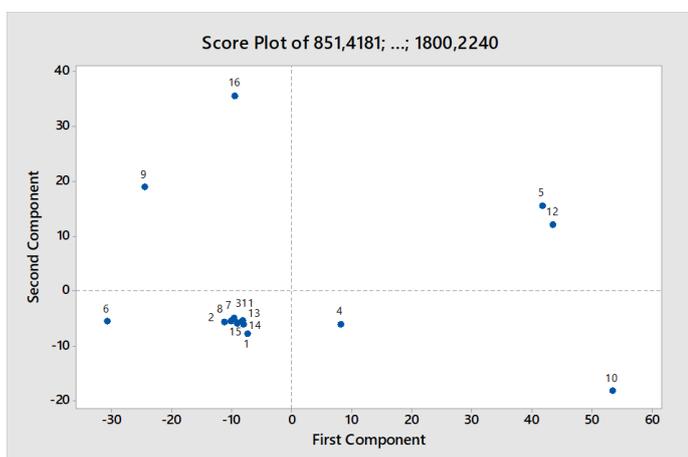


Figure 1. The PCA score plot of essential oil *C. mangga* Val. (16) and other oils. 1 = garlic oil; 2 = extra virgin olive oil; 3 = corn oil; 4 = castor oil; 5 = citrus oil; 6 = caraway oil; 7 = soybean oil; 8 = coconut oil; 9 = candlenut oil; 10 = cananga oil; 11 = sunflower oil; 12 = rose oil; 13 = palm oil; 14 = sesame oil; 15 = olive oil.

Figure 2 exhibits FTIR spectra of essential oil of *C. mangga* Val. (EOCM) and candlenut oil (CNO) scanned at wavenumbers of 4000-650  $\text{cm}^{-1}$  using horizontal attenuated total reflectance (HATR) as a sampling technique. Both FTIR spectra exhibited some differences due to the main components in both oils are different.

EOCM was mainly composed of monoterpenes (Abbas *et al.*, 2019), while CNO was typically composed of triglycerides (Jimenez-Carvelo *et al.*, 2017).

Each peak in certain frequency (wavenumbers) in FTIR spectra corresponded to functional groups which absorb IR radiation. The peak at 3007 is due to the stretching vibration of *cis* C=CH, while the peak at 2953  $\text{cm}^{-1}$  and 2920 originate from the asymmetric stretching vibrations of methyl (-CH<sub>3</sub>) and methylene (-CH<sub>2</sub>) groups. The peaks at 2873  $\text{cm}^{-1}$  come from symmetric stretching vibration of methyl (-CH<sub>3</sub>) group. The carbonyl (C=O) stretching vibration was observed at 1744  $\text{cm}^{-1}$ , while the peak at 1640 is from the stretching vibration of C=C. The bending vibrations of methylene and methyl were observed at wavenumbers of 1446 and 1378  $\text{cm}^{-1}$  respectively. The peak at 1105  $\text{cm}^{-1}$  comes from C-O vibrations. While, peaks at 988 and 892 originate from the bending out of plane vibrations of -HC=CH- (*trans*) and -HC=CH- (*cis*), respectively (Arslan *et al.*, 2019).

FTIR spectroscopy is considered as fingerprint analytical method, therefore, this technique is ideal for differentiation and quantification of analytes of interest in the samples. Quantitative analysis of EOCM in binary mixtures with CNO was performed using optimized FTIR spectra regions facilitated with multivariate calibrations of partial least square (PLS) and principle component regression (PCR). Both PCR and PLSR are inverse regression in which concentration of analytes (*y*-axis) was modelled with predictors of principle components, a linear combination of absorbance values of FTIR spectra of evaluated samples (*x*-axis) (Manaf *et al.*, 2007). PCR performs multiple inverse calibration of predictor variables against the scores or principle components (PCs) rather than original variables. PCR uses regression to convert PCs into concentration. PCs are obtained during principal component analysis (PCA). In addition, PLS finds the directions of greatest variability by comparing both spectral and target property information with the new axes, called PLS components. The optimum number of factors used to build PCR and PLS models are depending on the analytical target and properties of samples (Rohman and Putri, 2019).

FTIR spectra along with multivariate calibrations of PLS and PCR were optimized to get the prediction models between actual values of EOCM and FTIR predicted values. The parameters used to decide whether FTIR spectral regions and multivariate calibration chosen are based on the high coefficient of determination ( $R^2$ ) and low lowest root mean square of calibration

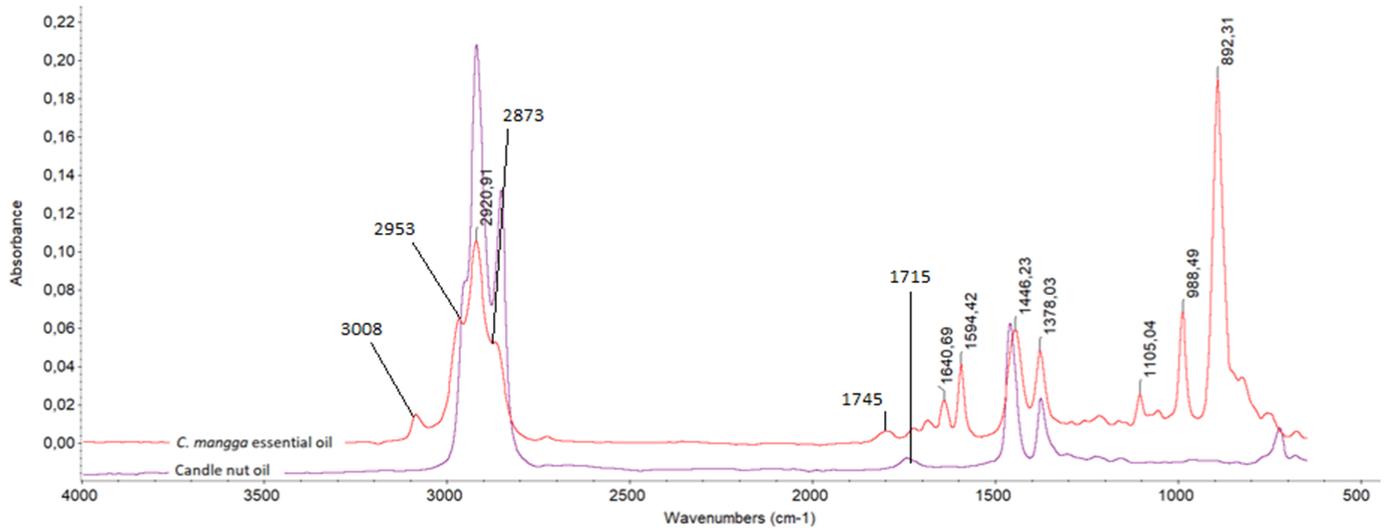


Figure 2. FTIR spectra of *C. mangga* Val. Essential Oil (CMEO) and Candle Nut Oil (CNO) scanned at mid infrared region (4000-650 cm<sup>-1</sup>) using attenuated total reflectance mode.

Table 1. The results of FTIR spectroscopy using different wavenumbers and spectral treatments coupled with multivariate calibration intended for prediction of *C. mangga* Val. Essential Oil (CMEO) levels adulterated with Candlenut Oil (CNO).

Multivariate calibrations	Wavenumber (cm <sup>-1</sup> )	Spectra	Calibration		Validation	
			R <sup>2</sup>	RMSEC	R <sup>2</sup>	RMSEP
PCR	3100-663	normal	0.9999	0.508	0.1	0.419
		derivative 1	0.9999	0.444	0.9999	0.494
		derivative 2	0.9999	0.413	0.9999	0.503
	1850-663	normal	0.9999	0.489	0.1	0.419
		derivative 1	0.9999	0.418	0.9999	0.499
		derivative 2	0.9999	0.39	0.9999	0.53
	3100-2700	normal	0.9998	0.55	0.1	0.374
		derivative 1	0.9998	0.601	0.9999	0.442
		derivative 2	0.9999	0.42	0.9999	0.533
	1614-1068	normal	0.9999	0.477	0.9999	0.463
		derivative 1	0.9999	0.484	0.9999	0.563
		derivative 2	0.9999	0.456	0.9999	0.484
	3100-2700 and 1850-663	normal	0.9998	0.543	0.9999	0.423
		derivative 1	0.9998	0.532	0.9999	0.447
		derivative 2	0.9999	0.442	0.9999	0.551
3100-2700 and 1614-1068	normal	0.9998	0.563	0.1	0.408	
	derivative 1	0.9998	0.577	0.9999	0.435	
	derivative 2	0.9999	0.509	0.9999	0.515	
PLSR	3100-663	normal	0.9999	0.544	0.1	0.391
		derivative 1	0.9999	0.476	0.9999	0.471
		derivative 2	0.9998	0.534	0.9999	0.465
	1850-663	normal	0.9998	0.531	1	0.341
		derivative 1	0.9999	0.483	0.9999	0.495
		derivative 2	0.9998	0.522	0.9999	0.549
	3100-2700	normal	0.9998	0.567	0.9999	0.452
		derivative 1	0.9999	0.382	0.9999	0.533
		derivative 2	0.9999	0.306	0.9998	0.724
	1614-1068	<b>normal</b>	<b>0.9999</b>	<b>0.485</b>	<b>0.9999</b>	<b>0.451</b>
		derivative 1	0.9999	0.496	0.9999	0.499
		derivative 2	0.9999	0.509	0.9999	0.502
	3100-2700 and 1850-663	normal	0.9998	0.543	1	0.401
		derivative 1	0.9998	0.53	0.9999	0.448
		derivative 2	0.9998	0.534	0.9999	0.458
3100-2700 and 1614-1068	normal	0.9998	0.575	1	0.346	
	derivative 1	1	0.263	0.9998	0.605	
	derivative 2	0.9999	0.437	0.9999	0.551	

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

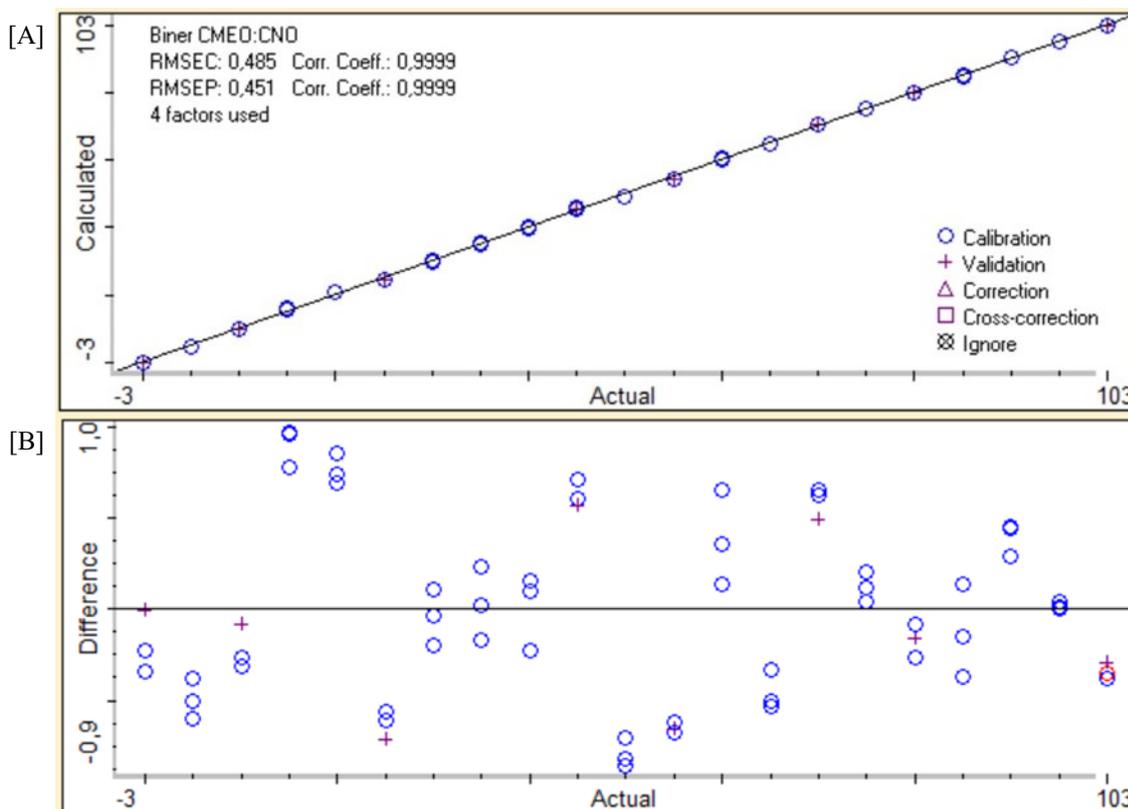


Figure 3. The relationship between actual values (x-axis) of *C. mangga* Val. Essential Oil (CMEO) in x-axis and the predicted values of CMEO using FTIR spectroscopy [A] along with residual analysis [B].

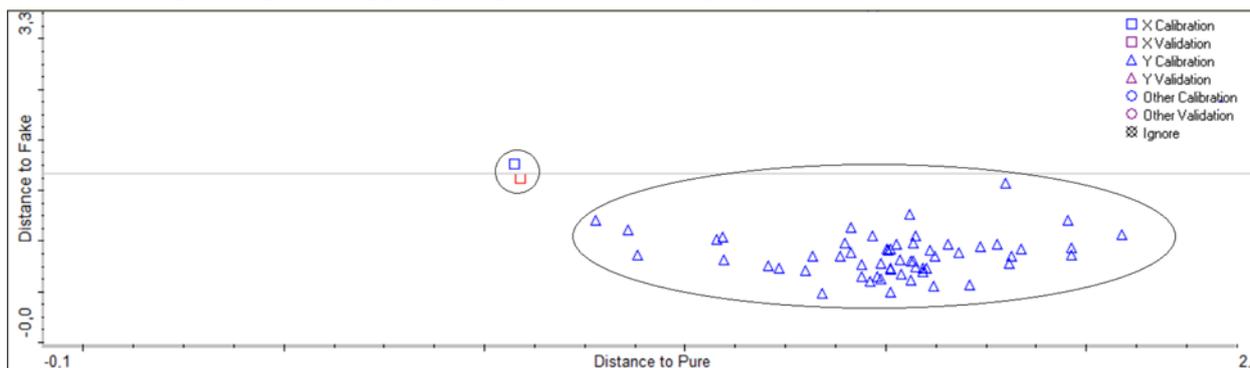


Figure 4. The Cooman's plot obtained during discriminant analysis for discrimination of pure *C. mangga* Essential Oil (CMEO) and CMEO mixed with Candle Nut Oil (CNO).

(RMSEC) and root mean square error of prediction (RMSEP).

Table 1 compiles the performance results of FTIR spectroscopy using different wavenumbers and spectral treatments coupled with multivariate calibration intended for prediction of EOCM levels adulterated with CNO. PLSR using normal FTIR spectra at wavenumbers of 1614-1068  $\text{cm}^{-1}$  was selected for the quantification of EOCM in CNO due to its capability to provide the highest  $R^2$  values (0.9999) either in calibration and prediction with lowest RMSEC value (0.485) and RMSEP value (0.451). The developed models revealed the acceptable accuracy and precision as shown by high values of  $R^2$  and low values of RMSEC and RMSEP. Figure 3A exhibited the linear relationship between actual values of EOCM (x-axis) and FTIR predicted values of EOCM (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 model developed are reliable to predict PSO adulterated with SeO.

Discriminant analysis (DA) is known as the supervised pattern recognition commonly used for discrimination between pure EOCM and EOCM adulterated with CNO (Rohman *et al.*, 2014). 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 Cooman's plot for classification between EOCM and EOCM adulterated with CNO successfully with accuracy level of 100%, without any misclassification.

#### 4. Conclusion

Fourier transform infrared (FTIR) spectroscopy in combination with chemometrics of multivariate calibration of partial least square regression (PLSR) and chemometrics of discriminant analysis was capable for the quantifying the levels of essential oil of *C. mangga* Val. (EOCM) adulterated with candlenut oil (CNO) at optimized wavenumbers of 1614-1068  $\text{cm}^{-1}$ . The model was valid as indicated by acceptable accuracy and precision as indicated by high  $R^2$  and low RMSEC and RMSEP. Discriminant analysis was also success for classification of EOCM and EOCM adulterated with CNO.

#### Conflict of Interest

The authors declare no conflict of interest.

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