

The employment of Fourier Transform Infrared Spectroscopy (FTIR) and chemometrics for analysis of candlenut oil in binary mixture with grape seed oil

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Abstract

Candlenut oil (CDO) is the target of adulteration with other plant oils to get economical profits, therefore, reliable analytical techniques should be developed. Based on the principal component analysis (PCA), grape seed oil (GSO) has the close similarity with CDO. Therefore, this study was intended to make modelling in the authentication analysis of CDO from GSO using Fourier transformed infrared (FTIR) spectroscopy in combination with chemometrics of partial least square calibration (PLSR) and discriminant analysis (DA). FTIR spectra of CDO, GSO and its binary mixtures were subjected to FTIR spectral measurement at wavenumbers of 4000-650 cm^{-1} , and its absorbances were used for modelling of PLSR and DA. FTIR spectra were also subjected to pre-processing including Savitzky-Golay derivatization. The optimization results showed that FTIR spectra using second derivative at the combined wavenumbers of 3000-2800 and 1600-650 cm^{-1} offered the optimum models. The coefficient determination (R^2) for the relationship between actual values and FTIR predicted values was 0.9996 and 0.9975 in calibration and internal validation (prediction) models, respectively. The errors in calibration and validation were relatively low, i.e. 0.84% and 2.19 %vol/vol, respectively. Using the same FTIR spectra, DA could discriminate pure CDO and that mixed with GSO at concentration range of 1-50%vol/vol. The combination of FTIR spectroscopy and chemometrics offered effective tools for the quantification and discrimination of CDO mixed with GSO with the main advantage of its simplicity and rapidity.

1. Introduction

Candlenut with the scientific name of *Aleurites moluccana* L., belonging to family of Euphorbiaceae is known with several names including *kemiri* in Indonesia, *Kukui nut* in Hawaii, and *buah keras* in Malaysia. This plant is flowering tree, typically having height up to 20 m (Norulaini *et al.*, 2004; Krisnawati *et al.*, 2011). The plant of *A. moluccana* is originating from the Indo-Malaysia region and the productivity of the oil yield obtained from its seed is approximately 3200 kg/ha annually. The leave's decoction of *A. moluccana* has been reported in folk medicines to treat diarrhea, coughs, headaches, hernia, ulcers, fever, and gonorrhoea (Quintãoa *et al.*, 2019), while candlenut oil has been applied topically to treat arthritis and other joint pain (Judd, 1998). Unfortunately, the seeds of plant are considered toxic for oral use (González-Stuart and Rivera, 2003). Candlenut oil (CDO), widely sold in cosmetics industry, has been used as good material for soap, varnish, and other oil-based cosmetics products.

Furthermore, the remaining seed cake after oil extraction can be exploited as fertilizer or animal fodder (Elevitch and Manner, 2006; Subroto *et al.*, 2017).

Due to its good properties to be used in pharmaceutical and cosmetics products, CDO has an expensive price in the oil industries and maybe target to be adulterated with other plant oils having lower price to get economical profit (Yuliani *et al.*, 2018). The authentication of high price oils such as CDO is interesting issue in fats and oils industry not only for economic reasons but also for health safety concerns (Abbas *et al.*, 2018). The vegetable oil authentication has been regarded as nice area of research and requires reliable analytical techniques to perform. The authentication analysis is usually carried out by fingerprinting profiling between authentic oil and adulterated oils. In addition, some authors used the major components such as triacylglycerol composition and fatty acid profiles as well as minor components such as tocopherols, carotenes, sterols, and volatile organic

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compounds to detect the oil adulteration (Milanez *et al.*, 2017).

Numerous analytical methods have been employed for authentication purposes based on molecular biology and physico-chemical properties such as chromatographic based techniques through analysis of major components of fatty acids and triacylglycerol compositions (Blasi *et al.*, 2019) and minor components like tocopherol contents (Cserhati *et al.*, 2005; Bosque-Sendra *et al.*, 2012), visible spectroscopy (Ferreiro-González *et al.*, 2011), synchronous fluorescence by analyzing the specific components capable of exhibiting photo-luminescence (Poulli *et al.*, 2007; Ge *et al.*, 2014), Ultrafast 2D nuclear magnetic spectroscopy (NMR) in combination with principal component analysis (Gouilleux *et al.*, 2018), ambient mass spectrometry using direct analysis in real-time (Vaclavik *et al.*, 2009) and vibrational-based spectroscopy such as infrared and Raman spectroscopy by applying specific wavenumbers corresponding wavenumbers specific to analyte(s) of interest (El-Abassy *et al.*, 2009; Rohman and Che Man, 2012). Among these techniques, Fourier transformed infrared spectroscopy (FTIR) spectroscopy is the most commonly used techniques for authentication of edible oils.

Combined with multivariate regression of partial least square and classification of principal component analysis (PCA) and discriminant analysis (DA), FTIR spectroscopy has emerged a powerful means for authentication of high price edible oils such as detection of hazelnut oil as adulterant in olive oil (Beaten *et al.*, 2005), authentication analysis of walnut oil from soybean oil (Li *et al.*, 2015), detection and quantification of soybean oil in binary mixture with cold-pressed black cumin seed oil (Arslan *et al.*, 2019), and ternary mixture with black cumin seed oil and corn oil (Rohman and Ariani, 2013). Using literature review, only one publication reporting the authentication of candlenut oil (CDO) from palm oil and corn oil. CDO has similar color with GSO which make the authentication analysis using color difference is rather difficult so that the use of instrumental analysis such as FTIR spectrophotometer is highly needed. This study applied FTIR spectroscopy coupled with chemometrics for authentication of CDO from grape seed oil having close FTIR spectra based on score plot principal component analysis.

2. Materials and methods

2.1 Materials

A total of twenty nine edible and non-edible oils namely cumin oil, rice bran oil, sunflower seed oil, coconut oil, pumpkin seed oil, sesame oil, candlenut oil,

Dayak onion oil, soybean oil, extra virgin olive oil, olive oil, grape seed oil, canola oil, castor oil, palm oil, corn oil, garlic oil, cananga oil, rose oil, orange oil, fannel oil, clove oil, eucalyptus oil, peppermint oil, paraffin liquid and aring oil were obtained from different markets around Yogyakarta. The other solvents and reagents were of pro-analytical grade.

2.2 Preparation of candlenut oil

Candlenut oil was prepared by direct pressing of candlenut seed using designed apparatus in Figure 1. The seed of candlenut was placed on the top of apparatus and the temperature was set at 80°C. The apparatus works as follows: when the temperature has reached 80°C, the seed of candlenut was pressed until the oil is separated from the pulp. Candlenut oil (CDO) obtained exhibited light yellow oil in color. CDO obtained was further used for the authentication studies using FTIR spectroscopy.

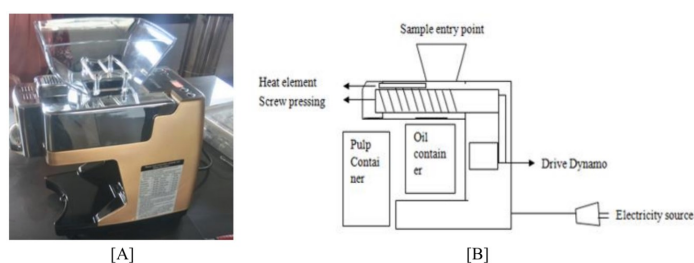


Figure 1. Apparatus used for pressing seed of candlenut to obtain candlenut oil.

2.3 Principal component analysis

Principal component analysis (PCA) was used for searching the oil having close similarity with CDO to make adulteration model. A total of twenty-nine samples of edible and non-edible oils were analyzed using PCA. As independent variables, absorbances at several wavenumbers were used and the responses investigated were score plot at first principle component (PC1) and second principle component (PC2). The closer PC1 and PC2 value, the more similar of oils based on used variables. PCA was carried out using Minitab software version 17 (Minitab Inc., USA).

2.4 Preparation of calibration and validation samples

Based on PCA results, grape seed oil (GSO) has the close PC1 and PC2 values with CDO, therefore GSO was used as oil model for adulteration analysis. For making calibration samples, twenty-six samples with a concentration range of CDO and GSO 0-100% were prepared by mixing both oils using calibrated pipette. For validation samples, a set of independent samples was also prepared. All samples were scanned using FTIR spectrophotometer.

2.5 Discriminant analysis

Discriminant analysis (DA) is one of the supervised pattern recognitions. During preparing of DA models, pure CDO and CDO mixed with GSO at the level range of 0.5–50.0% (volume/volume) was prepared. Pure PSO was marked as pure. In addition, CDO mixed with GSO was marked as adulterated. Both classes (pure CDO and adulterated samples) were subjected to DA using variables of Mahalanobis distance based on absorbance values at optimized wavenumbers region.

2.6 FTIR spectra acquisition

The FTIR spectra of all studied samples were scanned using 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 cm^{-1} with 32 scanning and the resolution was 8 cm^{-1} using horizontal attenuated total reflectance (HATR) composed of ZnSe crystal. All FTIR spectra were corrected against FTIR spectrum of air as background. After every scan, a new reference air background spectrum was taken. These spectra were recorded as absorbance values at each data point in triplicate.

2.7 Statistical analysis

Chemometrics analysis of PCA was carried out using Minitab version 17.0 software (Minitab Inc., USA). The multivariate calibration of partial least square regression (PLSR) and discriminant analysis (DA) was performed using TQ Analyst software version 9 (Thermo Fisher Scientific, Inc.). PLSR is one of inversed multivariate calibration in which concentration of analytes (y-axis) was modelled with absorbance values (x-axis) at certain wavenumbers.

3. Results and discussion

The first step for authentication study of candlenut oil (CDO) with other oils, principal component analysis (PCA) was applied to search oils having close similarity with CDO based on absorbance values at whole mid-infrared region (4000–650 cm^{-1}). PCA is one of the unsupervised pattern recognition techniques commonly applied to classify the samples. Figure 2 shows PCA score plot of CDO and other oils in which CDO has close similarity with grape seed oil (GSO), therefore GSO was selected as model of oil adulterant. CDO and GSO also has the close similarity in fatty acid compositions in which Stearic (C18:0), oleic acid (C18:1) and linoleic (C18:2) acids were dominated fatty acids in both CDO and GSO (Che Man *et al.*, 2011; Yuliani *et al.*, 2018).

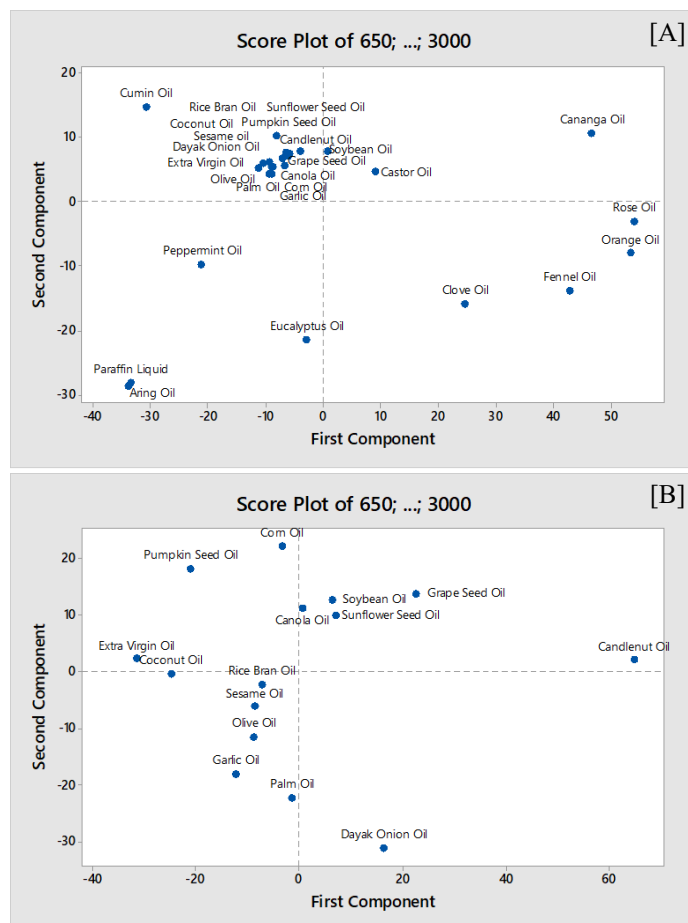


Figure 2. The score plot of principal component analysis (PCA) expressed by first principle component (PC1) and second principle component (PC2) for classification of 29 edible and non-edible samples [A] and 16 edible samples [B].

Figure 3 reveals the peaks and shoulders of FTIR spectra of CDO and GSO obtained at mid-infrared region (4000–650 cm^{-1}) representing the functional groups responsible for infrared absorption. The absorption of functional groups in each wavenumber fit the Lambert-Beer law, in which the intensity of each peak related with concentration, thus FTIR spectra could be effective means for authentication studies of edible fats and oils. Table 1 compiles the wavenumbers of each peak and shoulders in FTIR spectra of CDO and GSO and the corresponding functional groups' vibration (Rohman and Ariani, 2013). The functional groups composed CDO and GSO are basically similar to those in triacylglycerols (TAG), because fats and oils are basically composed of TAG (approximately 98%). Both spectra look very similar, but using detailed investigation there is a bit different in peak intensities at around 3000 cm^{-1} and at fingerprint regions (1500–650 cm^{-1}). Therefore, this difference could be target of optimization of FTIR spectra in terms of selection of wavenumbers region and spectral treatment (normal versus derivatization).

Partial least square calibration (PLSR) and principle component regression (PCR) were selected for prediction of CDO adulterated with GSO. PLS and PCR are inverse

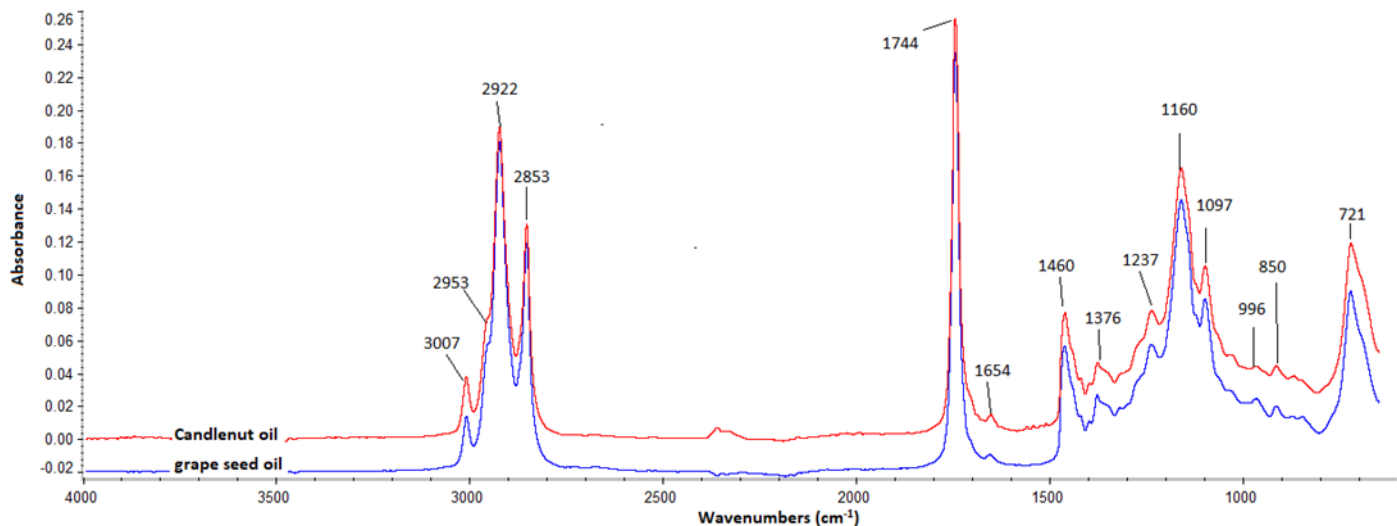


Figure 3. FTIR spectra of candlenut oil and grape seed oil scanned in mid infrared region corresponding to wavenumbers of 4000-650 cm^{-1} .

Table 1. The wavenumbers of FTIR spectra of candlenut oil and grape seed oil and the corresponding functional groups' vibration (Rohman and Ariani, 2013)

Wavenumber (cm^{-1})	Type of bending and vibration	Wavenumber (cm^{-1})	Type of bending and vibration
3009	C=CH Stretching	1235 and 1161	C-O (ester) Stretching
2922 dan 2852	-CH(CH ₃) Stretching Asymmetric	1118 dan 1098	C-O Stretching
1742	-C=O (ester) Stretching	964	trans-CH=CH-Bending out of plane
1658	-C=C (<i>cis</i>) Stretching	914	trans-CH=CH-Bending out of plane
1461	-C-H (CH ₂) Bending (scissoring)	871	trans-CH=CH-Bending out of plane
1378	-C-H (CH ₃) Bending symmetric	844	trans-CH=CH-Bending out of plane

regression in which absorbance values were used as independent variables. The absorbance values were combined to get new variables are known as principle components (in PCR) or PLS factors. Then, these factors were used for modelling the concentration of analytes. PLSR provide better predictive power over PCR (data are not shown), therefore, PLSR was then used for next optimization process. Table 2 compiles the performance of PLSR for quantitative analysis of CDO in binary mixture with GSO in calibration and validation models using different wavenumber regions and FTIR spectral treatment. The condition selected was relied on the capability to give the highest coefficient of determination (R^2) for the relationship between actual values of CDO and predictive values and the errors, expressed by root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP), in calibration and validation models. The R^2 value could be used as predictive the accuracy of developed model. The higher R^2 , the more accurate of the models. In addition, RMSEC and RMSEP described the difference between actual and predicted values using the selected model, therefore RMSEC and RMSEP expressed the precision of model. The lower RMSEC and RMSEP values, the more precise of the developed model. Based on the highest R^2 and the lowest errors, the second derivative FTIR spectra at the combined wavenumber of 3000-2800

and 1600-650 cm^{-1} offered the best models. The R^2 values were 0.9996 and 0.9975 in calibration and internal validation (prediction) models, respectively. This can be also stated that the accuracy of calibration and validation models was 99.96% and 99.75%, respectively. The errors in calibration and validation were relatively low, i.e. 0.84% and 2.19 %vol/vol, respectively (Figure 4[A]). The residual analysis confirmed that error occurred above and below the zero difference (as in Figure 4[B]), therefore the error occurring is random error, not systematic error.

In order to discriminate between CDO and CDO adulterated with GSO, discriminant analysis (DA) based on Mahalanobis distance was used. Using the same FTIR spectra (the second derivative FTIR spectra at the combined wavenumber of 3000-2800 and 1600-650 cm^{-1}), DA could discriminate pure CDO and that mixed with GSO at a concentration range of 1-50% vol/vol without any misclassification reported (Figure 5).

4. Conclusion

The combination of chemometrics of PLSR and discriminant analysis-second derivative FTIR spectra at combined wavenumber regions of 3000-2800 and 1600-650 cm^{-1} resulted in the effective analytical techniques for the quantification and discrimination of CDO mixed

Table 2. The performance of partial least square regression for quantitative analysis of candlenut oil in binary mixture with grape seed oil in calibration and validation models.

Wavenumber (cm ⁻¹)	Spectra	Factor	Calibration		Validation	
			RMSEC	R ²	RMSEP	R ²
4000-650	Normal	7	0.45	0.9999	2.21	0.9978
	Derivate 1	3	0.7	0.9997	2.36	0.9975
	Derivate 2	2	1.34	0.999	2.92	0.9961
3000-2800 and 1600-650	Normal	5	1.21	0.9992	2.17	0.9978
	Derivate 1	3	1.44	0.9988	2.48	0.9971
	Derivate 2	4	0.84	0.9996	2.19	0.9975
3050-2800	Normal	4	2.61	0.9962	2.97	0.9955
	Derivate 1	3	2.43	0.9967	2.66	0.9969
	Derivate 2	3	2.71	0.9959	3.15	0.9955
1800-650	Normal	3	2.26	0.9972	3.2	0.996
	Derivate 1	3	1.84	0.9981	3.04	0.9958
	Derivate 2	3	1.8	0.9982	2.86	0.9962

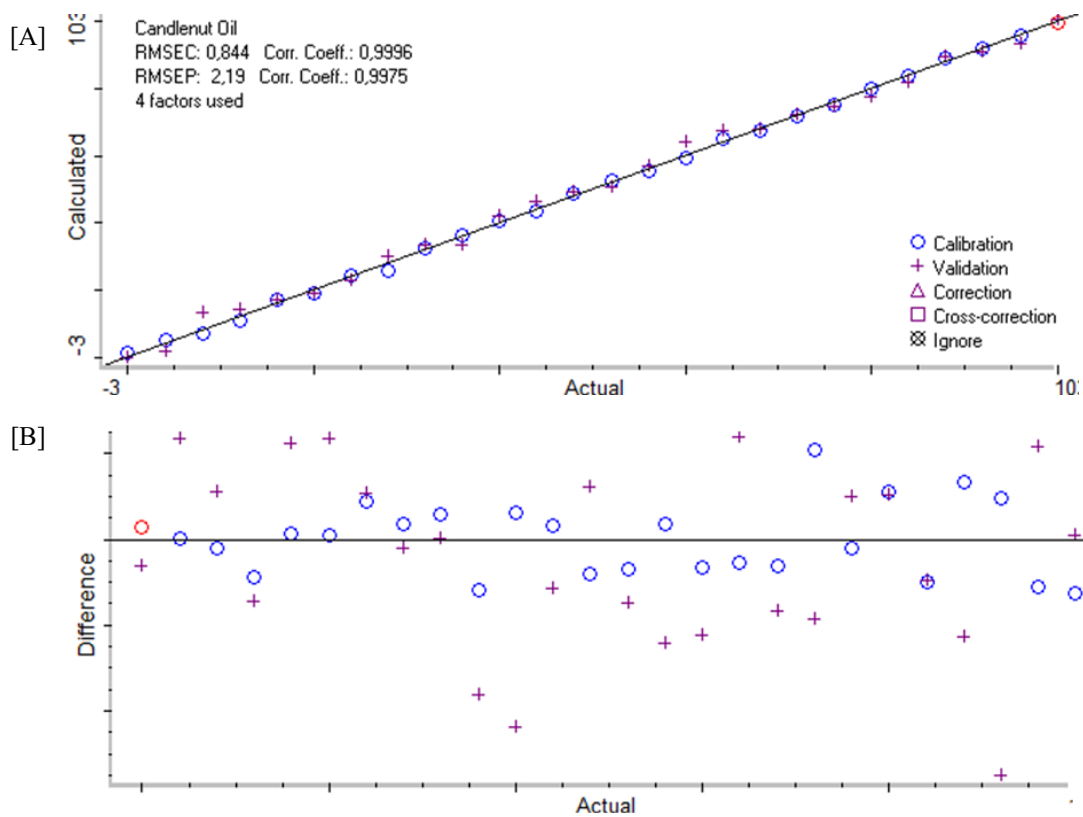


Figure 4. The relationship between actual values of candlenut oil in %volume/volume (x-axis) and FTIR predicted values in calibration and validation models [A] along with residual analysis [B].

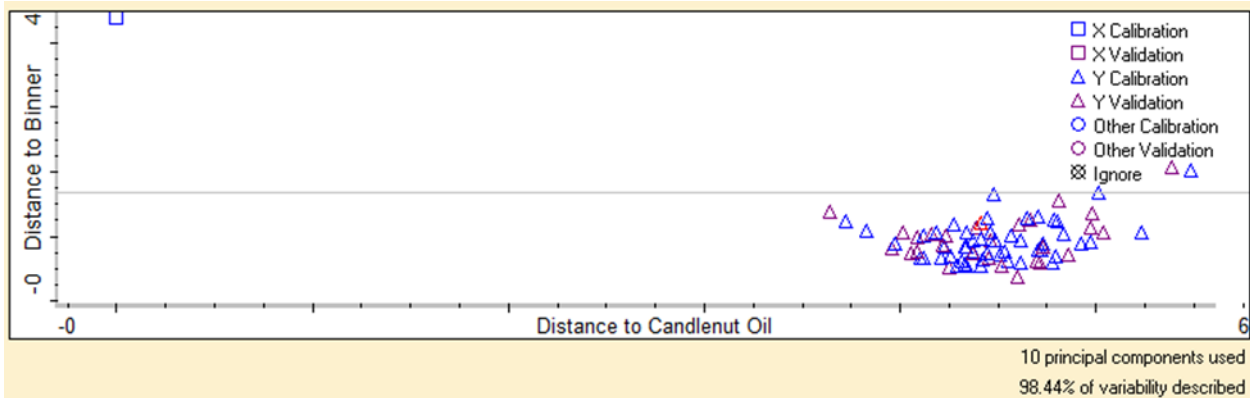


Figure 5. The Cooman's plot obtained during discriminant analysis for discrimination of pure candlenut oil and candlenut oil mixed with grape seed oil.

with GSO with acceptable accuracy as indicated by high R^2 values and acceptable precision with low RMSEC and RMSEP values. The developed method offered the main advantage of its simplicity and rapidity. Besides, FTIR spectra also promising analytical method for rapid screening of adulteration practice due to its nature as fingerprint analytical method.

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