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Original Article

APPLICATION OF FTIR SPECTRA COMBINED WITH CHEMOMETRICS FOR ANALYSIS OF CANDLENUT OIL ADULTERATION

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ABSTRACT

Objective: The aim of this study was to use FTIR spectroscopy in combination with chemometrics techniques for quantification and classification of candlenut oil (CnO) from oil adulterants, namely sunflower oil (SFO), soybean oil (SyO), and corn oil (CO).

Methods: The spectra of all samples were scanned using Fourier Transform Infrared (FTIR) Spectrophotometer using attenuated total reflectance (ATR) as sampling technique at mid infrared region (4000-650 cm^{-1}). Multivariate calibrations of principle component regression (PCR) and partial least square regression (PLSR) were used for quantitative models to predict the levels of CnO in the binary mixtures with SFO, SyO, and CO.

Results: The results showed that CnO in SFO was best quantified using PCR at wavenumbers region of 3100-2800 cm^{-1}. Quantitative analysis of CnO in SyO was carried out using PLSR with normal spectra mode using combined wavenumbers of 1765-1625 and 839-663 cm^{-1}, while CnO in CO was analyzed quantitatively using normal spectra at wavenumbers of 970-857 cm^{-1}. The coefficient of determination (R^2) obtained were>0.99 with low values of root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP). The results of discriminant analysis revealed that authentic CnO can be discriminated from CnO adulterated with SFO, SyO and CO using selected wavenumbers.

Conclusion: FTIR spectroscopy combined with chemometrics could be used as rapid and reliable method for authentication of candlenut oil (CnO) adulterated with other oils.

Keywords: Infrared spectroscopy, Chemometrics, Patchouli oil, Adulteration

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INTRODUCTION

Aleurites moluccana L., widely known as "kemiri" in Indonesia, "buah keras" in Malaysia, "candlenut" in English and "Kukui nut" in Hawaii is a flowering tree with up to 20 m in height, belongs to the family of Euphorbiaceae [1]. This plant is native to the region of Indo-Malaysia and become one of the world's great domesticated multipurpose trees. Candlenut oil (CnO) obtained from the seeds was taken into account as excellent material in the industries of cosmetics, soap, and pharmaceuticals [2]. It is estimated that the annual production of CnO is approximately 3200 kg/ha [3].

The price of candlenut oil (CnO) in the fats and oils industry, especially in Indonesia, is 10 times higher than that of other common edible oils like sunflower oil (SFO), soybean oil (SyO) and corn oil (CO), therefore, some unethical seller try to blend CnO either unintentionally or intentionally with cheaper oils to get economical profits [4]. Therefore, analytical methods capable of detecting the adulteration practice of high priced oils like CnO must be developed. Several methods based on physico-chemical and molecular biological properties have been reported for authentication of fats and oils. Such methods are differential scanning calorimetry [5], electronic nose [6], high-performance liquid chromatography with several detectors [7, 8], gas chromatography [9], capillary electrophoresis [10], nuclear magnetic resonance [11] and real-time polymerase chain reaction [12]. These methods are complex in instrumentation and need skilled analyst. For this reason, rapid quantitative analysis based on spectroscopic methods such as Fourier transform spectroscopy (FTIR) should be continuously developed.

FTIR spectrophotometry measures the functional group vibrations as a consequence of electromagnetic radiation with sample to generate a spectrum with fingerprint properties [12]. This techniques offer qualitative and quantitative analyses of targeted samples including pharmaceutical products [13, 14].

FTIR spectroscopy, especially in combination with chemometrics techniques, is one of the vibrational spectroscopic methods commonly used for rapid authentication analysis [16]. FTIR spectroscopy coupled with chemometrics have been used for authentication of extra virgin olive oil [17, 18], black seed oil [19], and avocado oil [20, 21], sesame oil adulterated with corn oil [22], and authentication of virgin coconut oil [23]. However, using literature searching there are no publication reports regarding the application of FTIR spectroscopy for authentication of candlenut. Therefore, the objective of this study was to use FTIR spectroscopy in combination with chemometrics for authentication of candlenut oil (CnO) with soybean oil (SyO), sunflower oil (SFO) and corn oil (CO).

MATERIALS AND METHODS

Materials

The seeds of *A. moluccana* was cut into small to fasten drying process and to increase the yield of oil. A-250 gram of cut seeds was dried into conventional oven at 90 °C for 90 min. The seeds were pressed such a way that its oil was extracted. The candlenut oil obtained was then filtered and centrifuged to separate oil from the impurities. Soybean oil, sunflower oil, corn oil, coconut oil, argon oil, peanut oil, rice bran oil, extra virgin olive oil, palm oil and sesame oil were purchased from supermarket around Yogyakarta, Indonesia. All chemicals and reagents used were of pro-analytical grade purchased from E. Merck (Darmstadt, Germany).

Analysis of fatty acid composition

Fatty acid composition of candlenut oil and oil adulterants (sunflower, soybean and corn oils) were analyzed using gas chromatography with flame ionization detector (GC-FID). Before being injected into GC-FID, the oil samples were subjected to derivatization using sodium methoxide and BF_3. The analytical procedure and GC-FID condition for analysis of fatty acid composition can be seen in Che Man *et al.* [24]. The identification of fatty acid was performed based on retention times of corresponding fatty acids present in samples with those in standards of fatty acid methyl esters from Sigma (Aldrich, USA). Quantification of each fatty acids was carried using internal normalization technique as in Nurrulhidayah *et al.* [22].

Quantitative analysis of candlenut oil using FTIR spectroscopy

Quantitative analysis of candlenut oil (CnO) in binary mixture with soybean oil (SyO), sunflower oil (SFO) and corn oil (CO) was facilitated with two multivariate calibrations, namely principle component regression (PCR) and partial least square regression (PLSR) by developing prediction models, either in calibration or validation. The calibration samples were prepared by mixing CnO with SFO, SyO and CO in the concentration range of 0-100%. For validation model, another independent samples with similar composition were also prepared. All samples (calibration and validation) were scanned using FTIR spectrophotometer.

Classification of CnO and CnO adulterated with other oils (SFO, SyO and CO)

Qualitative analysis of CnO adulterated with other edible oils (SFO, SyO and CO) was carried out by making a classification between CnO and CnO adulterated with SFO, SyO and CO using discriminant analysis (DA). During DA, a training sets of pure CnO and CnO adulterated with SFO, SyO and CO at concentration range of 1-50% was prepared. All CnO and CnO mixed with SFO, SyO and CO were assigned as "authentic" and "adulterated", respectively.

FTIR spectra measurement

The measurement of FTIR spectra of samples were done using FTIR spectrophotometer (Nicolet 6700 from Thermo Nicolet Corp., Madison, WI) equipped with detector of deuterated triglycine sulphate (DTGS). This instrument was connected to computer operating systems which include OMNIC operating system (Version 7.0 Thermo Nicolet) software. The sampling compartment was horizontal Attenuated Total Reflectance kit (HATR, Smart ARK, Thermo Electron Corp.) composed of ZnSe crystal. FTIR spectra were scanned at wavenumbers of 4000–650 cm^{-1}, using 32 scans with 4 cm^{-1} resolution, as absorbance mode.

Data analyses

TQ Analyst™ software version 6 from Thermo electron Corporation (Madison, WI, USA) was used during data analysis, including multivariate calibrations and discriminant analysis. FTIR spectral regions where the variations existed were chosen for analysis.

RESULTS AND DISCUSSION

For selection of edible oils which are potential as adulterants in CnO, principal component analysis (PCA) was used. PCA is one of chemometrics techniques commonly used for the classification of oils with similar chemical properties. PCA was performed using absorbance values at 11 wavenumbers of 3009, 2923, 2853, 1743, 1657, 1463, 1377, 1241, 1159, 1097, 915, and 721 cm^{-1}. Fig. 1 revealed PCA score plot of CnO and other vegetable oils, representing the projection of evaluated samples defined by PC 1 (the first principle component) and PC 2 (the second principle component). PC1 and PC2 showed the most variation among variables used during PCA. Based on PC1 and PC2 scores, soybean oil (SyO), sunflower oil (SFO) and corn oil (CO) have the closer distance to CnO, indicating the close similarity between CnO with SFO, SyO and CO in terms of FTIR spectra, therefore, SFO, SyO and CO were selected as oil adulterants in CnO.

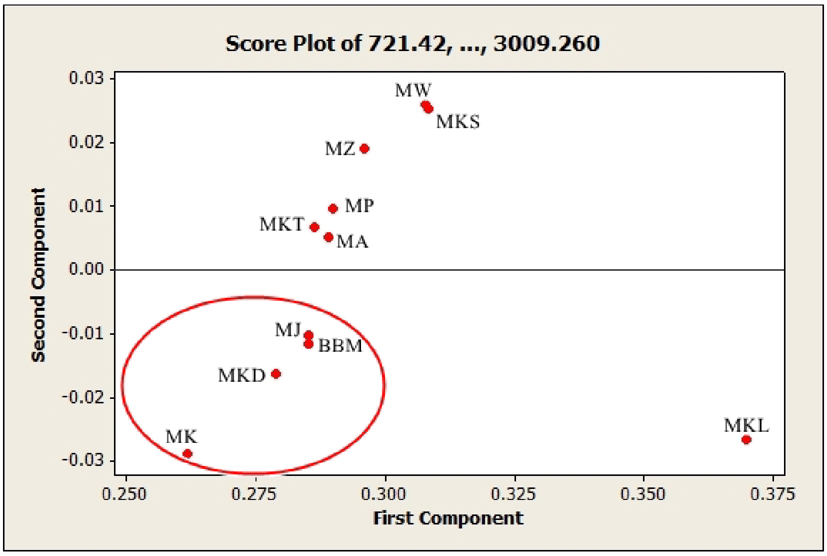

Fig. 1: The PCA sore plots of first principle component (PC1) and second component (PC2) of candle oil (CnO) and other edible oils. SyO = soybean oil; SFO = sunflower oil; CO = corn oil; CoO = coconut oil; ArO = argon oil; PeO = peanut oil; RBO = rice bran oil; OO = olive oil; PaO = palm oil; and SeO = sesame oil

In order to assure that the used oil adulterants were not mixed with other oils, fatty acid composition was used for its characterization. Stearic (C18:0), linoleic (C18:2) and oleic acids were main fatty acids composed CnO accounting of 42.28, 26.32, and 23.12%, respectively. In addition, linoleic, oleic and palmitic acids were dominant fatty acids composed SFO accounting of 67.80, 19.05, and 6.27%, respectively. Linoleic and oleic also main fatty acids composed corn oils, i.e. 59.08 and 25.86%, respectively. SyO was characterized by linolenic acid accounting of 8.05%. These fatty acids are in agreement with those appeared in Norulaini *et al.* [25] and Che Man *et al.* [23]. This indicated that the used oils were not added with other oils.

Fig. 2 revealed FTIR spectra candlenut oil, sunflower oil, corn oil and soybean oil scanned using FTIR spectroscopy at mid infrared region (4000-650 cm^{-1}) which were characteristic to triacilglyceride of infrared absoption. The interpretation of each peakas can be seen in Lerma-Garcia *et al.* [15] and Rohman [13]. The main functional groups were: (a) cis CH=CH stretching vibration appeared in wavenumbers (1/λ) of 3008 cm^{-1}, (b)–CH_3 stretching vibration in 1/λ of 2953 cm^{-1}, (c)–CH_3 stretching vibration in 1/λ of 2953 cm^{-1}, (d) very intense absorption of carbonyl (C=O) stretching vibration at 1/λ of 1741 cm^{-1}, (e) 1460 cm^{-1} corresponding to CH_2 bending, (f) 1378 cm^{-1} due to CH_3 bending, while (g) at 1235 cm^{-1}, (h) at 1159 cm^{-1}, (i) at 1118 cm^{-1}, (j) at 1098 cm^{-1} were corresponding to C-O stretching. All these functional groups are present in triglyceridic compounds.

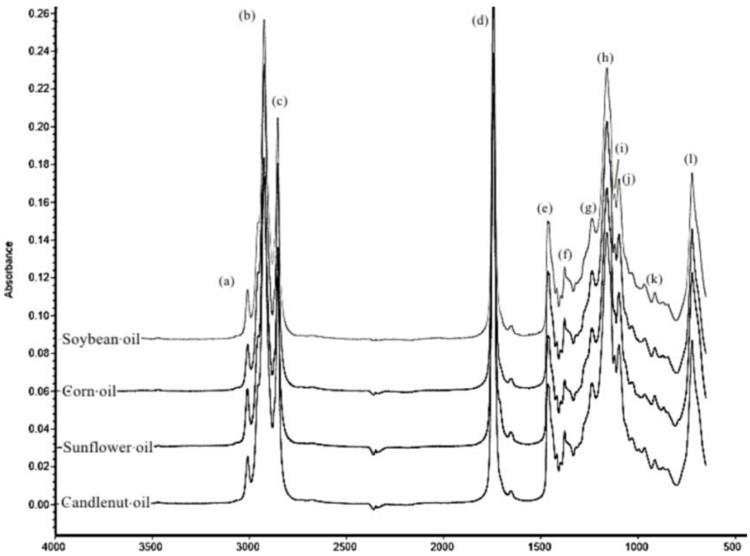

Fig. 2: FTIR spectra of candlenut oil, sunflower oil, corn oil and soybean oil scanned using FTIR spectroscopy at mid infrared region (4000-650 cm^{-1})

Table 1: The performance of multivariate calibrations along with wavenumbers and FTIR spectral treatments for analysis of candlenut oil (CnO) in the binary mixtures with sunflower oil, soybean oil, and corn oil

Oil adulterans	Multivariate calibration	Wave-numbers (cm^{-1})	Treatments*	Factor	Calibration		Validation	
					R²	RMSEC	R²	RMSEP
Sunflower oil	PCR	4000-650	Normal	10	0.9984	1.72	0.9982	2.28
			Der. 1	10	0.9985	1.71	0.9986	2.46
			Der. 2	10	0.9910	4.12	0.9904	7.51
		3100-2800	***Normal***	***10***	***0.9990***	***1.52***	***0.9985***	***1.56***
			Der. 1	10	0.9978	2.78	0.9992	1.50
			Der. 2	10	0.9961	2.99	0.9966	2.53
		1500-1000	Normal	10	0.9987	1.54	0.9975	2.66
			Der. 1	10	0.9986	1.65	0.9977	2.44
			Der. 2	10	0.9978	2.04	0.9981	2.24
	PLS	4000-650	Normal	6	0.9989	1.47	0.9976	2.56
			Der. 1	3	0.9995	0.99	0.9984	2.49
			Der. 2	7	0.9999	0.06	0.9926	6.98
		3100-2800	Normal	5	0.9989	1.58	0.9983	1.72
			Der. 1	3	0.9987	1.71	0.9987	1.65
			Der. 2	2	0.9964	2.87	0.9960	2.76
		1500-1000	Normal	6	0.9989	1.47	0.9976	2.56
			Der. 1	2	0.9974	2.20	0.9980	2.41
			Der. 2	6	0.9999	0.499	0.9978	2.51
Soybean oil	PCR	1765-1625; 839-663	Normal	10	0.9994	1.05	0.9989	1.63
			Der. 1	10	0.9958	2.77	0.9970	3.11
			Der. 2	10	0.9705	7.29	0.9435	11.8
		956-899	Normal	10	0.9981	1.86	0.9981	2.15
			Der. 1	10	0.9960	2.70	0.9963	3.35
			Der. 2	10	0.8514	15.9	0.8424	17.7
	PLS	1765-1625; 839-663	***Normal***	***5***	***0.9996***	***0.867***	***0.9990***	***1.61***
			Der. 1	5	0.9994	1.02	0.9968	2.73
			Der. 2	7	0.9986	1.59	0.9845	6.50
		956-899	Normal	2	0.9978	1.99	0.9979	2.14
			Der. 1	1	0.9953	2.92	0.9966	3.47
			Der. 2	9	0.9911	4.03	0.9682	7.78
Corn oil	PCR	970-857	Normal	10	0.9968	2.11	0.9883	4.83
			Der. 1	10	0.9867	4.25	0.9839	5.08
			Der. 2	10	0.7636	16.9	0.6569	21.6
		970-838; 1125-1111	Normal	10	0.9959	2.32	0.9946	4.39
			Der. 1	10	0.9899	3.63	0.9913	4.55
			Der. 2	10	0.9766	5.50	0.9692	9.70
	PLS	970-857	***Normal***	***3***	***0.9959***	***2.38***	***0.9958***	***2.63***
			Der. 1	9	0.9992	1.04	0.9820	5.65
			Der. 2	3	0.9483	8.31	0.7683	18.5
		970-838; 1125-1111	Normal	4	0.9939	2.82	0.9927	4.36
			Der. 1	5	0.9975	1.81	0.9909	5.21
			Der. 2	7	0.9985	1.38	0.9839	8.58

*treatments marked with bold are selected for the modelling calibration and validation

Quantitative analysis of candlenut oil

Quantitative analysis of candlenut oil (CnO) in binary mixture with SFO, SyO and CO was facilitated with multivariate calibrations of principle component regression (PCR) and partial least square regression (PLSR). Both regressions are based on inverse regression in which concentrations in y-axis are modelled as function of combination of variables of absorbances called as principle components in x-axis (Miller and Miller, 2010). Using these multivariate calibrations, several statistical parameters namely coefficient determination (R^2), root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) were used for evaluation of calibration and validation models. In this study, the models was optimized by selecting the best FTIR spectral regions and spectral treatments (derivatization) capable of providing the highest R^2 value and the lowest RMSEC and RMSEP.

Table 1 compiled the performance of PCR and PLSR for quantification of CnO in SFO, CO and SyO in terms of R^2, RMSEC, and RMSEP values using several wavenumbers region and spectral treatments (normal, first derivate and second derivate). Based on highest R^2 either in calibration and validation samples and lowest RMSEC and RMSEP values obtained, CnO in SFO was quantified using PCR at wavenumbers region of 3100-2800 cm^{-1} using FTIR normal spectra With R^2 calibration of 0.9990, R^2 validation of 0.997, RMSEC of 1.52% and RMSEP of 1.56%. In addition, CnO in SyO was analyzed using PLSR with normal spectra in the combined wavenumbers of 1765-1625 and 839-663 cm^{-1}. Finally, the normal spectra at wavenumbers region of 970-857 was selected for quantification of CnO in binary mixture with CO.

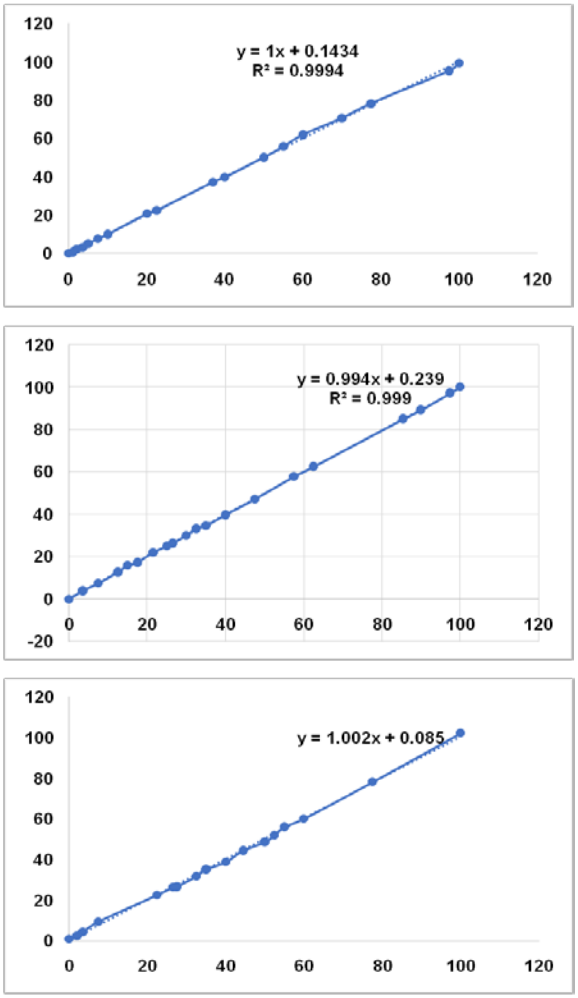

Fig. 3: The calibration model describing the relationship between actual values and FTIR predicted values of candlenut oil in not sunflower oil (top), soybean oil (middle), and corn oil (down)

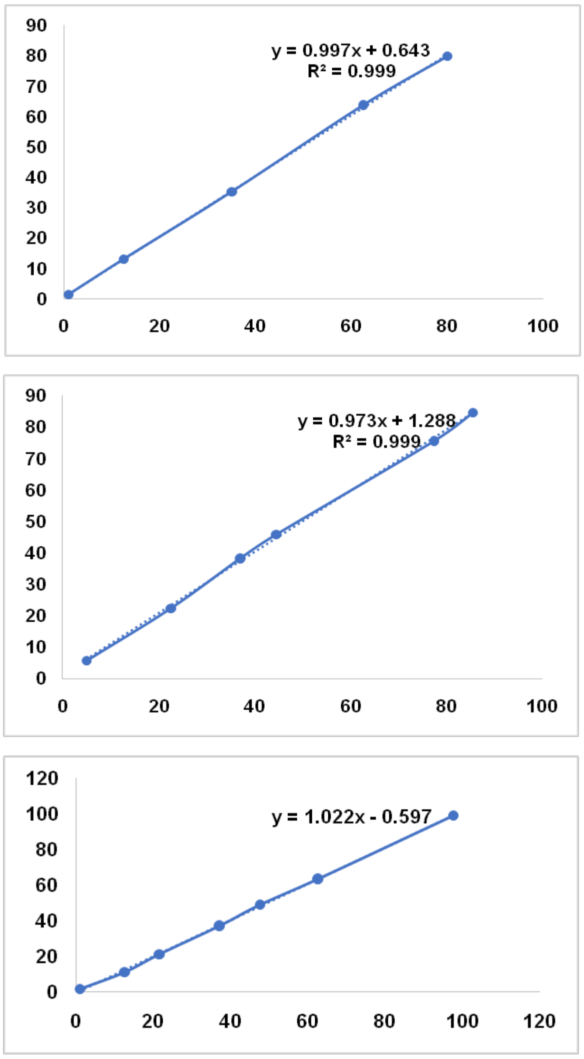

Fig. 4: The calibration model describing the relationship between actual values and FTIR predicted values of candlenut oil in not sunflower oil (top), soybean oil (middle), and corn oil (down)

Fig. 3 and fig. 4 revealed the relationship between actual values and FTIR predicted values of CnO in SFO, SyO and CO along with its equations in calibration and validation models, respectively. The residual point (the difference between actual and predicted) was around zero, indicating that no systematic errors were observed during modelling, therefore the calibration and validation models developed were reliable for quantification of CnO in the mixture mixtures with SFO, SyO and CO.

Discriminant analysis

In order to make classification between authentic CnO and CnO adulterated with other vegetable oils (SFO, SyO and CO), discriminant analysis (DA) was used. DA is one of supervised pattern recognition technique based on linear discriminant function, which is a linear combination of the original measured variables [26]. During classification using DA, the absorbances at selected wavenumbers regions was chosen such a way that they provide clear separation between two-classes of samples. Fig. 5 revealed the Coomans plot for discrimination between CnO and CnO adulterated with SFO (using absorbance at wavenumbers of 3100-2800 cm^{-1}), SyO (at 1765-1625 cm^{-1} and 839-663 cm^{-1}), and with CO (at wavenumbers of 1125-1111 cm^{-1}). The accuracy levels of 100% were obtained meaning that misclassification did not occur. Sometimes, if the studied oils have close similarity between authentic and adulterated oils, misclassification may occur, and then the model of DA must be re-optimized.

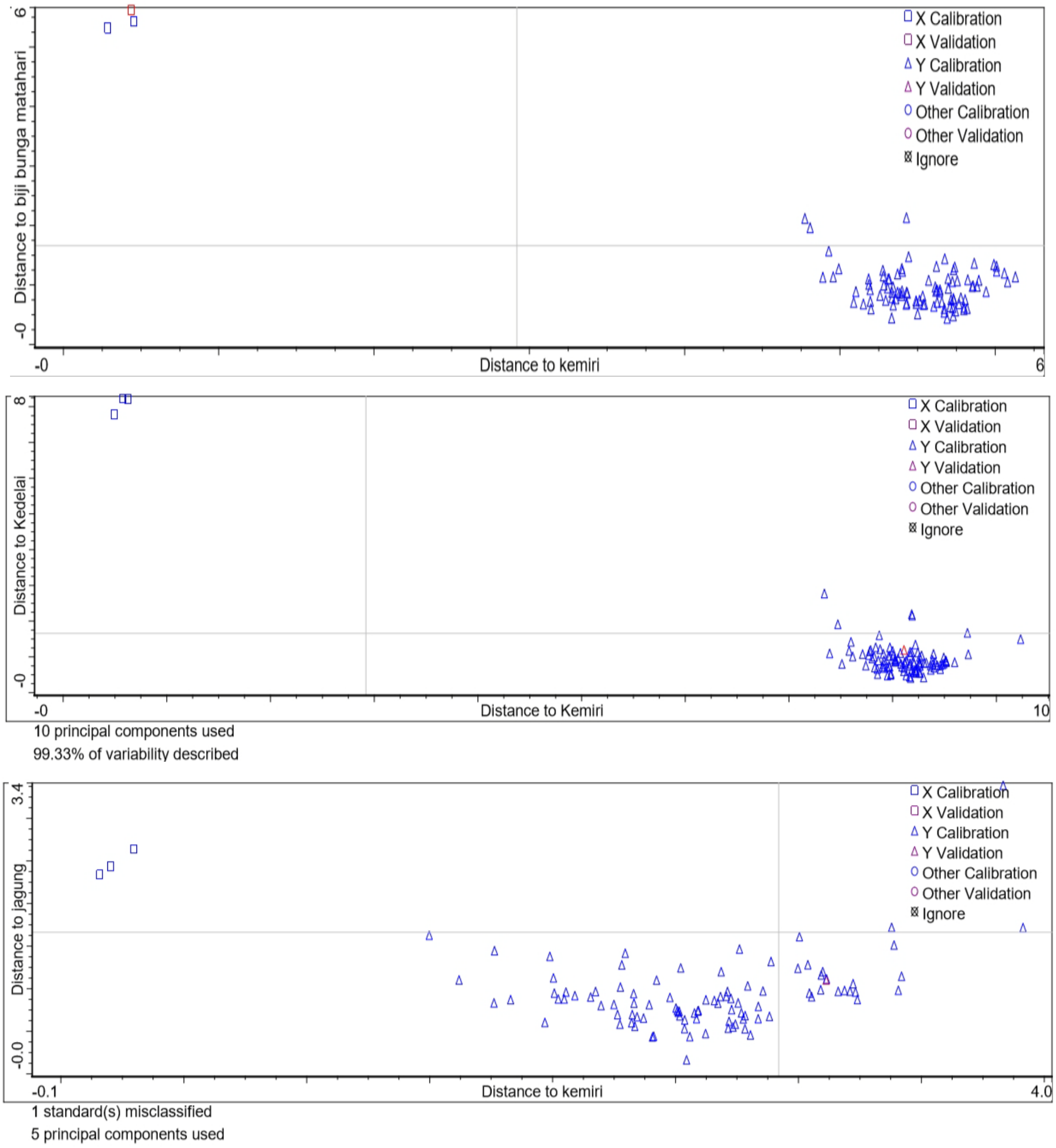

Fig. 5: The Coomans plot for the classification of candlenut oil (□) and candlenut oil adulterated with other oils (△), namely sun flower oil (top), soybean oil (middle), and corn oil (down)

CONCLUSION

The combination of FTIR spectroscopy with chemometrics of multivariate calibrations (PCR and PLSR) and discriminant analysis using absorbance values as variables at optimized wavenumbers regions are powerful analytical technique for authentication analysis of candlenut oil (CnO) from sunflower oil, soybean oil, and corn oil. Discriminant analysis allow rapid classification between CnO and CnO adulterated with other vegetable oils with accuracy levels of 100%. The developed method was reliable, rapid, and ease in sample preparation, which make it as rapid tools for quality control of edible oils.

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AUTHORS CONTRIBUTIONS

FY and AR performed research activities and prepared manuscript. SR and AR designed research, analyzed data, and made critical thinking on manuscript.

CONFLICTS OF INTERESTS

All authors have none to declare

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