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Rapid determination of adulteration in virgin and copra coconut oil using Fourier transform near infrared spectroscopy

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Abstract
The purity of coconut oil is important in assuring the benefits to the consumers as well as to the food industry. In the study, palm olein adulteration in copra coconut oil (CCO) and Virgin coconut oil (VCO) was respectively quantified by Fourier Transform near Infrared (FT-NIR) Spectroscopy combine with partial least square (PLS) model. NIR spectral variations due to difference in fatty acid content of coconut and palm olein was used to develop the PLS model. Discriminant analysis was done in order to classify adulterated and pure coconut oil in the Coonman plot. The levels of adulteration in blind VCO and CCO samples were estimated using PLS calibrations and unknowns could be analyzed in ±2.20% and ±2.64% accuracy respectively for VCO and CCO samples. This demonstrates that FT-NIR method can be used to detect adulteration of coconut oils using palm olein with sufficient accuracy and precision.

Keywords: Virgin coconut oil, copra coconut oil, palm olein, partial least square, fourier transform near infrared spectroscopy

1. Introduction

Coconut (*Cocos nucifera*) cultivation is widely spread in South East Asian countries where tropical weather conditions are prevailing. Coconut oil is one of the main coconut base products manufactured and used extensively in the region. It is basically produced from copra or in the form of virgin coconut oil (VCO) by wet or dry method [1].

Copra coconut oil (CCO), refined de-order bleached coconut oil and VCO are produced in Sri Lanka in larger manufacturing scale, but CCO is the most popular type among the Sri Lankan consumers. But due to reported health benefits such as cholesterol lowering capacity [2], antimicrobial activity against gram positive bacteria and a number of fungi and viruses [3], cardiovascular lowering effect [4], use as the remedy to Alzheimer disease [5], its favorable aroma and availability of most beneficial natural compound due to no chemical and no high heat treatments during manufacturing process [6] consumer's interest have increased on, VCO. Because of increasing demand for coconut oil is tend to be adulterated by cheap oils such as palm olein for unscriptural financial gains. Thus, the development of a reliable rapid technique is desirable to assess the quality of the oils available for the consumers.

Several techniques have been developed to detect coconut oil adulteration which include gas chromatography [7], high performance liquid chromatography [8], thin layer chromatography and colorimetric method [9]. These methods are time consuming and expensive and hence not suitable to handle large number of samples originating and distributed to the consumer market. FTIR based rapid techniques have developed to authenticity analysis of edible oils [10, 11, 12, 13] but the FTIR method includes pre sample preparation procedures. NIR methods do not suffer from the cumbersome sample preparation procedures. This is due lack of sample thickness problem in NIR region which is common with the Mid Infrared (MIR). Therefore, it does not require sample dilution or KBr pallet preparation techniques. Hence, NIR is a non-distractible technique [14] and the analysis can be completed in less than one minute. Also, non-destructive NIR technique allows multiple analyses from a single sample permitting the monitoring the manufacturing process at different stages with no harm to the product. Thus, NIR technique is user friendly, environmental benign, cost effective, less cumbersome and rapid than the MIR technique and it is ideal in assessing the quality of the coconut oils.[15]

NIR region covers 14,000cm^{-1}to 4000 cm^{-1}wavenumbers which includes combinations and overtones of CH, CH_2, OH, COO moieties in the sample. Normally, NIR spectra have broad overlapping bands, which may not reveal the qualitative information directly. However, with the development of chemometrics, data handling from NIR spectra is getting easier and adaptable. With the software development it is possible to exploit small changes associated with spectra with leads to differentiation of each other [16].

Several NIR base methods have recorded for adulteration determination in edible oils[16], but there are no records for palm olein adulteration determination method for copra and virgin coconut oil. Therefore, this research work is a novel work.

The key objective of this work is to develop FT-NIR base technique with combination of chemometric analysis method to determine palm olein adulterated amount in CCO and VCO. The outcome of study can be used to ensure the consumer's trust on CCO and VCO produce in the country.

2. Materials and methods

2.1 Sample preparation

Palm olein was purchased from local market. CCO and VCO were prepared under controlled conditions in the laboratory. Matured coconuts (10-11month maturity) were purchased from Coconut Research Institute of Sri Lanka. In order to prepare CCO coconut meat was peeled with testa and sundried for three weeks. For VCO coconut meat was scraped without testa and dried in 58(±1) °C. Both types of coconut oil were extracted by extruding dries coconut via small scale expeller. The obtained oil was centrifuged in 10,000 rpm for 20 min to obtained clear oil.

2.2 Preparation of adulterated series

A standard series of adulterated samples were prepared by mixing CCO with palm olein. For the analysis twenty adulterated samples ranging from 1% v/v to 70% v/v of palm olein in CCO was prepared by vortexing at 1500 rpm for 2 min. The adulteration points in the adulteration series was prepared in three point intervals from 1% to 40% and five points intervals starting from 40% to 70%. Each adulterated sample was duplicated. The same procedure was followed for VCO.

2.3 Obtaining NIR spectra

NIR spectra obtained using spectral transmittance in the wavenumber range of 12,000 – 4000 cm^{-1} by Thermo Scientific Antaris Π NIR spectrophotometer using the Omnic software supplied with FT-NIR instrument. For each sample, 32 replicate scans were done with a resolution of 4 cm^{-1}.

2.4 Chemometric analysis

The NIR spectra were subjected to multivariate analysis. The optimum calculation method was done by partial least square (PLS) using TQ analyst software supplies by Thermo Scientific Antaris Valpro software package. The cross validation was done by leaving one sample out at a time to determine minimum number of factors used for calibration. Both calibration and validation, calculated against actual curves were plotted and regression coefficient (R^2) and root mean squared error (RMSE) for calibration and validation were calculated respectively. Blind samples prepared by intentionally adulterating palm olein respectively with VCO and quantified using the developed model. The same procedure was done for CCO.

2.5 Discriminate analysis

Palm olein adulterated VCO samples in the range of 1-70% were prepared to obtain 20 adulterated VCO samples. Same process was done for CCO. Pure VCO and CCO sample were prepared in the laboratory according to the definitions of SLS 32:2017 standard. Discriminate analysis (DA) was done for palm olein adulterated VCO samples and pure VCO samples. In the DA classification was done based on FT-NIR spectra. Further, attempt was made to classify adulterated VCO with adulterated CCO samples by DA.

3. Results & Discussion

3.1 Quantification of CCO & VCO adulteration by Palm olein

Figure 1, illustrates the FT-NIR spectra for pure VCO, CCO and Palm olein in the range of 10,000 – 4500 cm^{-1}. According to the spectra, three main spectral band regions can be identified, in the ranges of 4700-6000 cm^{-1}, 6800-7400 cm^{-1} and 8000-9000 cm^{-1} and there is no significant bands found in wavenumber greater than 9000 cm^{-1}. Assignments of relevant NIR bands to each bonding are tabulated in Table 1.The combination of NIR bands around 4700-6000 cm^{-1} are for CH 1st overtone, $CH_2$1st overtone, CH_3 1st overtone and CH_3 combination. In the other two regions CH_3, CH_2 and CH 1st overtone combinations and their second overtones can be identified respectively.

Coconut oil contains saturated fatty acids with short and medium chain lengths fatty acids while palm olein is having unsaturated medium chain fatty acids as the major fatty acid constituent. Both coconut oil and palm olein contain Capric ($C_{10:0}$), Lauric ($C_{12:0}$), Myristic ($C_{14:0}$) and Palmitic ($C_{16:0}$) as saturated fatty acids. Coconut oil contains Caproic ($C_{6:0}$) and Capyllic ($C_{8:0}$) as short chain fatty acids in addition to above mentioned saturated fatty acids. Palm olein contains monounsaturated oleic ($C_{18:1}$), diunsaturated linoleic ($C_{18:2}$) and poly unsaturation linolenic ($C_{18:3}$) while coconut oil only contains unsaturated fatty acids of monounsaturated oleic ($C_{18:1}$) and diunsaturated linoleic ($C_{18:2}$). From all the fatty acids coconut oil has lauric acid as the major fatty acid while palm olein contains unsaturated Oleic acid as the major fatty acid. Therefore, unsaturated fatty acid quantity is higher in palm olein in compared to coconut oil. This is reverse in saturated fatty acid content [17, 11]. Because of above mentioned variations of chemical composition, the spectra of two oils vary. But as same functional groups such as CH, CH_2, OH are available in different constituent in two oils, it is impossible to identified specific peaks which is specific to one oil but the absorbance variation of two oils exhibit these chemical differences which can be exploited for the authentication. The notable variations can be recognized in the circled area in Figure 1 from 5800 to 4500 cm^{-1} region of the spectra.

CH 1st overtone, CH_2 1st overtone, CH_3 combination bands are representing in the spectral region of 5800 to 4500 cm^{-1}. CCO and VCO spectra have relatively low absorbance with comparing to palm olein. This is due to higher amount of unsaturation in palm olein. Therefore, this region is used to develop multivarious calibration. This variation is not much prominent for both CCO and VCO in the overtone regions and therefore this specific region is used to develop multivarious calibration. With increasing of palm olein adulteration respectively the absorbance at corresponding NIR regions are increased. This principle can be used to develop multivarious calibration method to determine palm olein adulteration amount.

PLS is more accurate and widely used method for chemometrics analysis with comparing to PCR and other classical methods [18]. Therefore PLS was used as the chemometric analytical method for this study. The PLS calibration and validation plots for VCO and CCO are given in Figure 2. The regression coefficient (R^2) for calculated concentrations against actual concentration plots of VCO was 0.9997. The validation was done by leaving one sample out at a time, and R^2 after validation was 0.9993. Since the R^2 values are close to one it proves PLS calculated values are well correlated with the actual values. The root mean square error (RMSE) for calibration and validation were 0.5 % and 0.8 % respectively in VCO. The small values of RMSE indicated higher accuracy of the calculated data. Also, for CCO, R^2 for calibration and validation were 0.9994 and 0.9990 respectively, and RMSE were 0.5 % and

0.6 %. These results also indicated that the high correlation between PLS calculated and actual values of adulteration.

Table 2 figure out the single blind samples analyzed via developed methods for CCO and VCO respectively. The blind samples covered range from 3% to 65%. Concentration residue, difference between actual and detected value from developed method was determined to distinguish the accuracy of blind sample results. According to the Table 2, residue values were varied from 0.09% to 6.63% for VCO and 1.31% to 6.18% for CCO. The mean value and standard deviation of concentration residue are 2.29% and 1.52% for VCO. The mean value and standard deviation of concentration residue are 2.64% and 1.49% for CCO. Even though residue values vary throughout the analyzed blind sample range, the standard deviations of residue value are small for both CCO and VCO. Therefore it is possible to use average of concentration residue as the error for blind samples. Therefore the error for VCO sample analysis is ±2.20%, for CCO ±2.64%. These results indicated the FT-NIR method can be used to quantification of unknown palm olein adulterated samples in rapid cost effective manner.

3.2 Discriminate analysis (DA)

In this study discriminate analysis was used to discriminate pure coconut oils from adulterated oil coconut oil group in qualitative manner. The adulteration series used for PLS analysis in the palm oil bending range of 1-70 % and pure ten samples of CCO and VCO were used for DA.

Figure 3 a, demonstrate Cooman plot between the pure CCO and palm olein adulterated CCO and Figure 3 b, demonstrate the Cooman plot between VCO and palm olein adulterated VCO. The spectral range which issued for PLS analysis, 5800-4500 cm^{-1}was used to develop DA. The range was used due to previously described spectral variation because of the fatty acid composition variations in two oil types. DA was done using 10 principal components. The x-axis shows the Mahalanob is distance to the pure coconut oil while y-axis shows Mahalanob is distance to coconut oil adulterated by palm olein.

It can be observed a clear separation between palm olein adulterated samples and pure VCO samples in Cooman plot a. Also same thing was observed in CCO pure sample and palm olein adulterated CCO group in Cooman plot b. In the study there are no any misclassifications within these pure and adulterated oil groups. This clear separation of adulterated and pure coconut oil sample proves the possibility of classification of adulterated sample higher than 1% of palm oil adulteration. This clear classification is due to above mentioned fatty acid variation in between palm olein and coconut oil. The same discriminate analysis was done between palm olein adulterated VCO and palm olein adulterated CCO sample. The result of this DA is indicates in c Cooman plot of Figure 3. These two groups did not clarify into two groups. This may be due to similar nature of CCO and VCO which is the only difference in two classification groups.

In some studies misclassification has been reported and could be happened due to close similarities of these types of sample groups [12].

4. Tables and Figures

Table 1: Assignment of NIR band positions for NIR spectra

Functional group assignment	Spectrum region cm^{-1}
CH 1st overtone	6000 – 5500
CH2 1stovertone	6000 – 5400
CH3Combination band	4400 - 5200
CH21stOvertone combination	6800 - 7200
CH2 3rd Overtone	8200 - 8600

Table 2: Determination of sets of VCO and CCO blind samples from PLS calibration methods.

Level of adulteration %	Level of detected for VCO %	Concentration residue for VCO	Level of detected for CCO %	Concentration residue for CCO
3	2.18	0.82	5.54	2.54
7	5.46	1.54	8.31	1.31
11	9.98	1.02	9.48	1.52
13	11.43	1.57	11.88	1.88
15	12.24	2.76	11.90	3.10
20	17.50	2.50	13.82	6.18
22	19.59	2.41	16.60	5.40
25	23.36	1.64	21.05	3.95
27	26.37	0.63	24.77	2.23
30	29.17	0.84	27.72	2.28
32	31.38	0.64	29.33	2.67
35	36.01	1.01	32.66	2.34
37	37.91	0.91	35.17	1.83
40	39.91	0.09	37.78	2.22
42	40.42	1.58	36.98	5.02
45	47.35	2.35	42.04	2.96
47	49.86	2.86	44.33	2.67
50	52.94	2.94	46.70	3.30
52	55.74	3.74	51.87	0.13
55	59.70	4.70	52.68	2.32
57	59.73	2.73	52.80	4.20
60	66.63	6.63	57.99	2.09
62	66.26	4.26	61.88	0.12
65	67.54	2.54	64.01	0.99

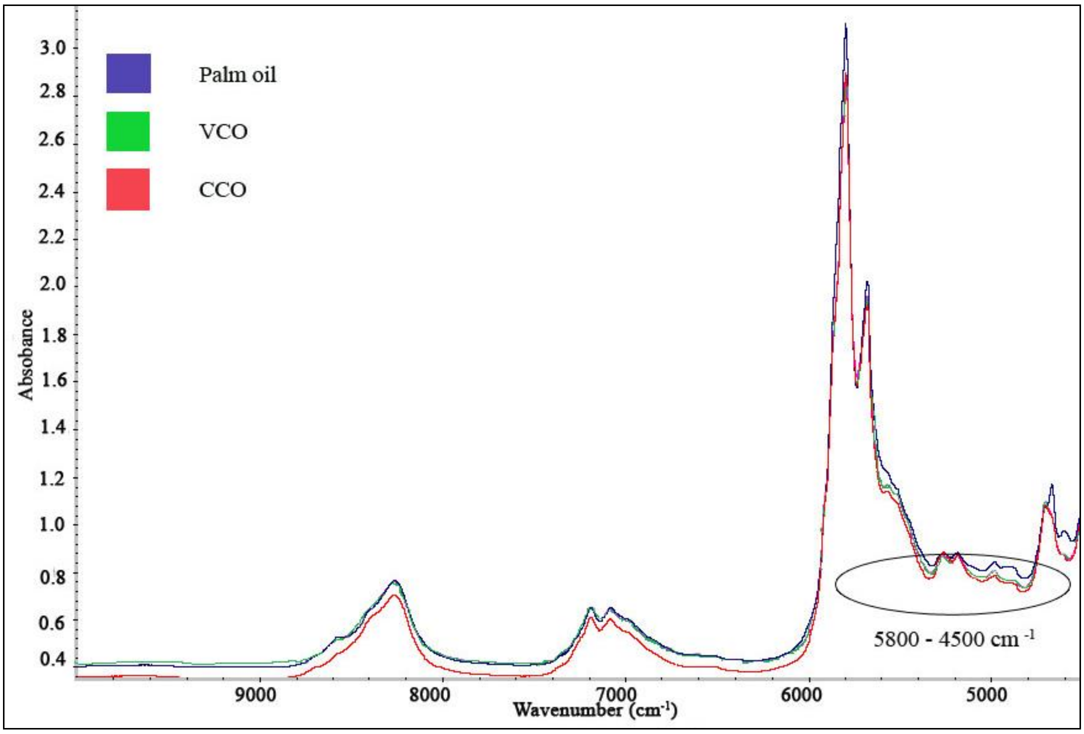

Fig 1: FT-NIR Spectra for virgin coconut oil, copra coconut oil and palm olein.

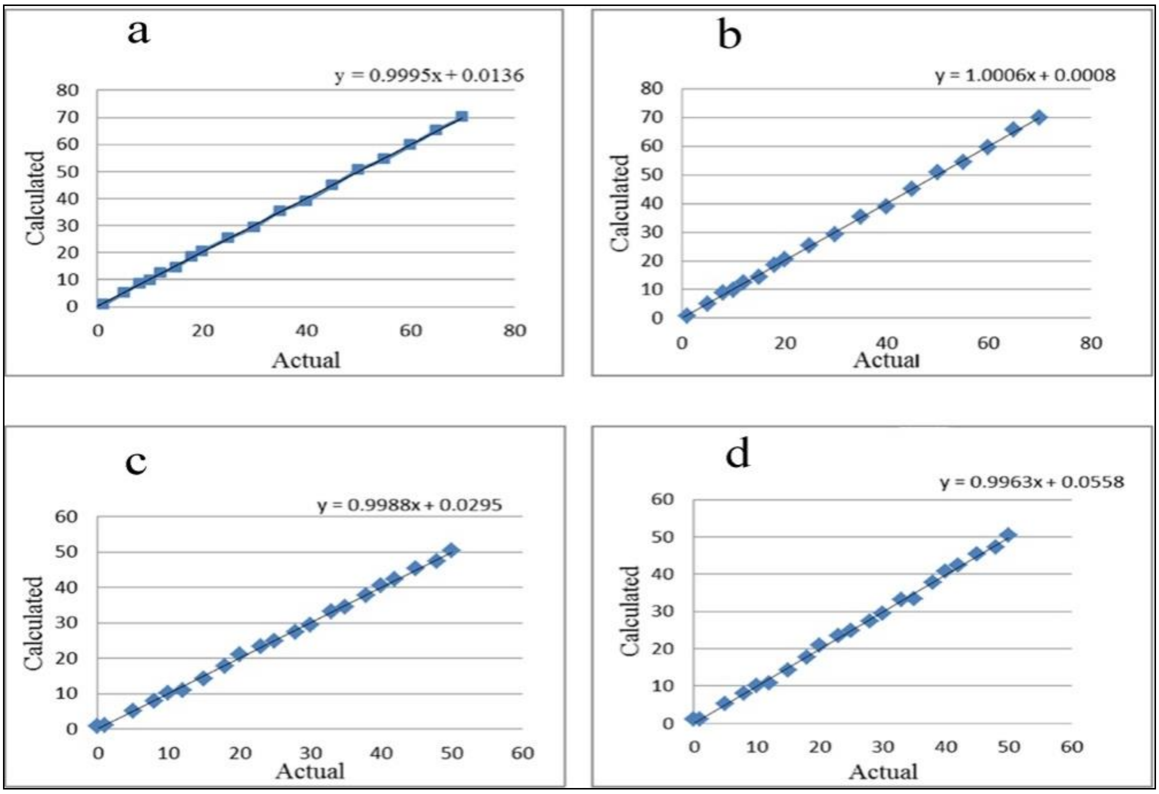

Fig 2a: PLS calibration curves for VCO, b. PLS validation curve for VCO, c. PLS calibration curves for CCO and d.PLS validation curve for CCO.

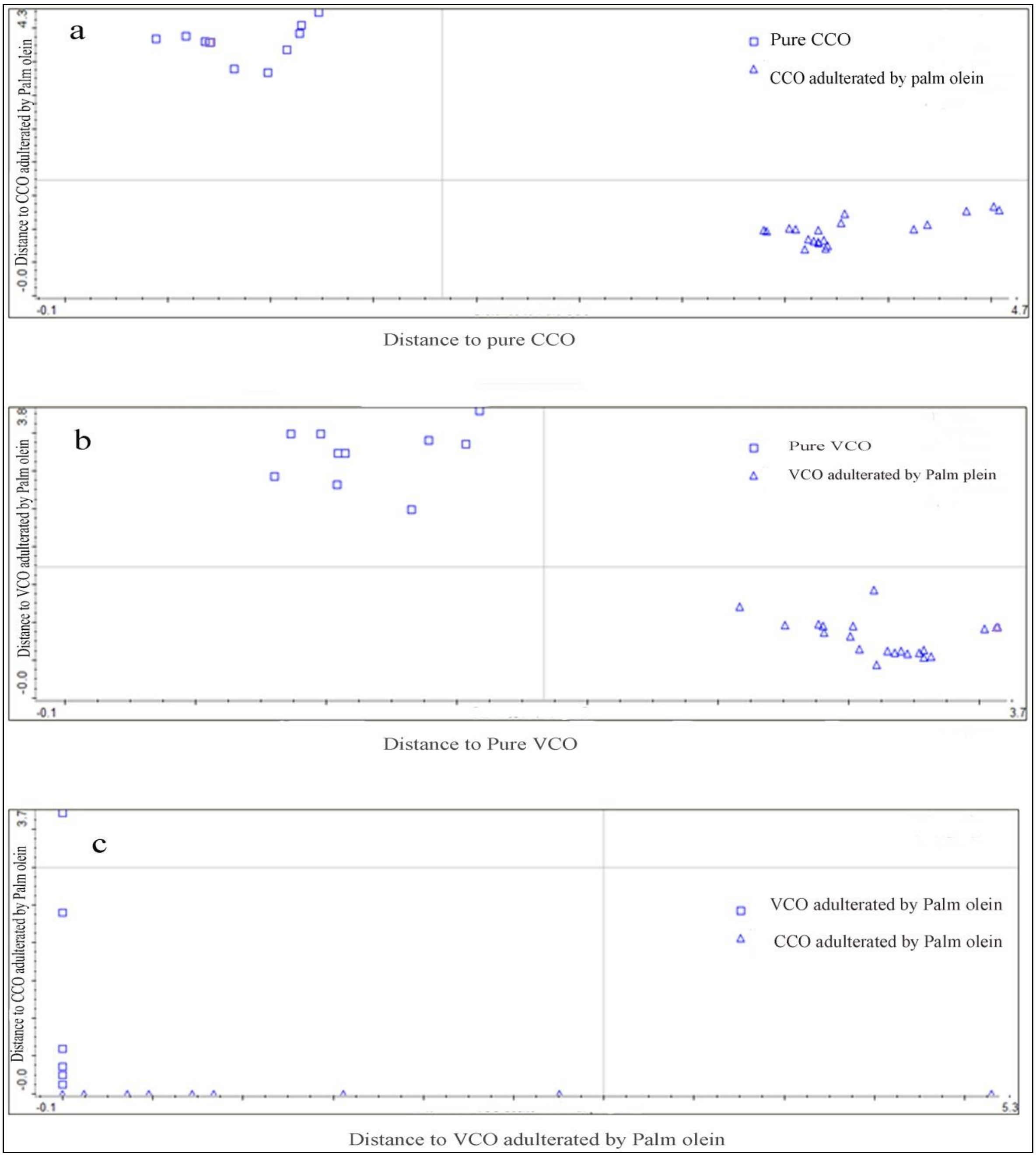

Fig 3 a: The Cooman plot for CCO adulterated by palm olein, b. Cooman plot for VCO adulterated by palm olein, c. Cooman plot for CCO adulterated by palm olein and VCO adulterated by palm olein

8. Conclusions

FT-NIR combination with multivariate analysis can be used to determine palm olein adulteration in virgin and copra coconut oil. These methods allow determining palm olein adulteration to virgin coconut oil with ±2.20% accuracy and ±2.64% accuracy for CCO. But it is impossible to classify CCO and VCO from each other from this method. Therefore, it needs further developments to classify these two oil types. Finally, discriminate analysis allows classifying pure coconut oil from palm olein adulterated coconut oil. The developed NIR based method is rapid, nondestructive environmentally friendly and cost-effective method; therefore it is beneficial to assess the quality of commercial coconut oil samples.

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