THE AUTHENTICATION OF VIRGIN COCONUT OIL FROM GRAPE SEED OIL AND SOYBEAN OIL USING FTIR SPECTROSCOPY AND CHEMOMETRICS

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ABSTRACT

Objective: The objective of this study was to develop Fourier transform infrared (FTIR) spectroscopy in combination with chemometrics of multivariate calibration and discriminant analysis (DA) for the authentication of virgin coconut oil (VCO) from grape seed oil (GSO) and soybean oil (SO).

Methods: FTIR spectra of VCO, GSO, SO and its binary mixture of VCO-SO, and VCO-GSO were scanned at mid-infrared region (4000-650 cm\(^{-1}\)) using attenuated total reflectance technique. The wavenumbers were selected based on its capability to provide the best prediction models for quantification and classification of adulterants in VCO assisted by multivariate calibrations and DA, respectively.

Results: The results showed that partial least square (PLS) calibration using absorbance values at combined wavenumbers of 1200-900 and 3027-2985 cm\(^{-1}\) revealed reliable method for quantification of GSO in VCO, as indicated by high value of coefficient of determination (R\(^2\)) and low value of root mean square error of calibration (RMSEC) and root mean square error of prediction (RMSEP). PLS using FTIR spectra at the combined wavenumbers of 1200-1000 and 3025-2995 cm\(^{-1}\) was suitable for quantitative analysis of SO in VCO. DA was also successfully used for classification of VCO and VCO added with adulterants of GSO and SO.

Conclusion: FTIR spectroscopy in combination with chemometrics of multivariate calibration and DA offered effective tools for the authentication of VCO.

Keywords: Virgin coconut oil, Chemometrics, Authentication, FTIR spectroscopy

INTRODUCTION

Virgin coconut oil (VCO), a tropical plant oil, is an emerging issue in fats and oil industry, especially in Southeast Asian region like Indonesia, Malaysia, and the Philippines due to its property to have some biological activities including antioxidant, anti-thrombotic, and anti-bacteria against Listeria monocytogenes [1,2]. VCO is rich in medium chain fatty acids (MCFA), especially lauric acid (C12:0), and exhibits good digestibility [3]. Combined with menhaden oil, VCO also exhibited the protective effect through reducing the incidence of the mammary tumor during the animal study [4]. VCO is extracted from the fresh and mature kernel of the coconut meat using either dry or wet methods [5], which retained the active compounds in it. Due to these reasons, VCO is more expensive than other edible oils such as palm oil, grape seed oil, and soybean oil which make VCO is more expensive than other edible oils.

The authentication of high-quality edible oils like extra virgin coconut oil (VOO) and VCO is an interesting study, due to the fact that adulteration practice is a crucial issue, not only for the consumer but also for producers and regulators in field fats and oils [7]. As a consequence, several physicochemical and molecular biology methods have been continuously developed for identification of adulteration practices of high-value fats and oils. Valid and reliable analytical methods have been reported for the authentication of VCO, namely fast gas chromatography [8], differential scanning calorimetry [9], electronic nose [10], nuclear magnetic resonance phosphorus-31 (31P NMR) spectroscopy [11], and two-dimensional gas chromatography (GC x GC) coupled with time flight mass spectrometry (TOF-MS) [12]. However, these methods required sophisticated instruments and need skillful analyst. To overcome these instrumental problems, some simple analytical techniques for authentication of high edible oils have been proposed, developed and standardized. One of the ideal methods providing fast and reliable results is Fourier transform infrared (FTIR) spectroscopy due to its property as the fingerprint technique, especially in combination with multivariate analysis (chemometric).

FTIR spectroscopy combined with some chemometrics techniques of multivariate calibration of partial least square and discriminant analysis have been successfully used for authentication of VCO from some lower-priced fats and oils, namely lard from palm kernel oil [13], VCO from palm oil [14], corn and sunflower oils [15]. FTIR spectroscopy in combination with chemometrics was also used for authentication of candlenut [16] and for analysis of fatty acids [17]. However, the application of FTIR spectroscopic method for authentication of soybean oil and grape seed oil having a different characteristic with those oils has not been reported yet. Therefore, the objective of this research was to develop FTIR spectroscopy in combination with chemometrics of multivariate calibrations and discriminant analysis for quantification and classification of GSO and SO in VCO for authentication purposes.

MATERIALS AND METHODS

Materials

The used oils in this study, namely virgin coconut oil (VCO), grape seed oil (GSO), and soybean oil (SO) with different brands were purchased from Serdang, Selangor, Malaysia. In order to assure the authenticity that the used oils were not blended (adulterated) with other oils, the composition of fatty acid (FA) in the oils were used to confirm its purity. The profiles of FA in the evaluated oils were compared with those specified in Codex Alimentarius [18]. The oils are considered as authentic if FA composition meets the FA specification stated in Codex Alimentarius. The solvents and reagents used during this study were of pro-analytical grade and purchased from E. Merck (Darmstadt, Germany).

Determination of fatty acid composition

The levels of fatty acids (FAs) in VCO, GSO and SO are determined by gas chromatography equipped with flame ionization detector (GC-FID), as described in our previous paper [14]. Fatty acids are not volatile so that they must be derivatized into their methyl esters to...
obtain fatty acid methyl esters (FAMEs) which are volatile before GC-FID measurement. Briefly, 1 ml oil sample was added with 4 ml of sodium hydroxide 0.5 M in methanol, heated for 20 min under nitrogen. The mixture was then added with 5 ml of boron trifluoride 5%, prepared freshly in methanol. After 2 min, 5 ml of heptane and 2 ml of saturated NaCl were added, shaken vigorously. Heptane phase (supernatant) was separated and added with anhydrous sodium sulfate. Supernatant containing FAMEs were separated using capillary column RTX-5 (0.25 mm internal diameter, 30 m length, and 0.2 µm film thickness; Restex Corp., Bellefonte PA). The temperature was programmed as 500C (hold for 1 min), increased to 2400C (8oC/min), and finally held at 2400C for 5 min. The temperatures of the detector and injector for 1 min), increased to 2400C (8oC/min), and finally held at 2400C for 5 min. The temperatures of the detector and injector for 1 min), increased to 2400C (8oC/min), and finally held at 2400C for 5 min. The temperature of analyser was programmed as 60°C (hold for 1 min), increased to 240°C (8°C/min), and finally held at 240°C for 5 min. The temperatures of the detector and injector were set at the same temperature as those of the analyser.

Identification of FA was carried out by comparing retention times of FAMEs in sample oils with those in a mixture of 37 FAME standards (FAMEs, C4 to C24) from Sigma Chemicals (St. Louis, MO, USA). While quantitative analysis of FA was performed using the normalization area (relative percentage). The analysis was done in three triplicates (n = 3).

### Quantitative analysis of grapeseed oil and soybean in VCO

Quantitative analysis of GSO and SO as adulterants in VCO was aided with multivariate calibration of principle component regression (PCR) and partial least square (PLS) regression. A set of 30 VCO samples containing GSO and SO in the concentration ranges of 1.0–5.0 % (v/v) was prepared and called with calibration samples. For preparing validation samples, 25 independent samples containing the mixture of VCO-GSO and VCO-SO with different concentrations were also prepared. The mixture of samples was shaken vigorously to assure homogenous samples before analysis using FTIR spectrophotometer.

### Discriminant analysis

Discriminant analysis (DA) is a supervised pattern recognition technique commonly used for classification among samples, was used to classify VCO and VCO mixed with GSO and SO (as adulterants). A set of 20 pure VCO (non-adulterated model) and 20 sets of VCO mixed with GSO and SO in the concentration range of 1-50% of GSO and SO (adulterated model) were also prepared. VCO and VCO mixed with GSO-SO were subjected to FTIR spectra measurement and classified using DA with the aid of TQ Analyst software.

### Measurement of FTIR spectra

The oil samples are scanned using FTIR spectrophotometer (Nicolet 6700 from Thermo Nicolet Corp., Madison, WI) facilitated with the detector of DTGS (deuterated triglycine sulphate) as a detector and connected to OMNIC operating system software (Version 7.0 Thermo Nicolet). The sampling compartment was Smart Attenuated Total Reflectance kit (Smart ARK, Thermo Electron Corp.) composed of zinc selenide (ZnSe) crystal. FTIR spectra of samples were measured at 4000–650 cm-1, using 32 scans with a resolution of 4 cm-1. These spectra were subtracted against air spectrum as the background. These spectra were recorded as absorbance mode at each data point in triplicate.

### Chemometrics and statistical analyses

The software TQ AnalystTM version 6 from Thermo Electron Corporation (Madison, WI, USA) included in FTIR spectrophotometer was used for data analysis which included the modelling of calibration and validation models based on multivariate calibration (PLS and PCR) and for classification using discriminant analysis (DA). The spectral regions where the variations were observed were selected and optimized during analysis (PLS, PCR and DA).

### Table 1: Fatty acid composition in virgin coconut oil (VCO), grapeseed oil (GSO), and soybean oil (SO)

| Fatty acids | Virgin coconut oil | Grape seed oil | Soybean oil |
|------------|--------------------|----------------|-------------|
| C8:0       | 7.8±0.36 (4.6-10.0)| nd             | 0.05±0.00 (na)|
| C10:0      | 6.08±0.31 (5.0-8.0)| 0.05±0.00 (na) | 0.09±0.00 (nd-0.1)|
| C12:0      | 47.01±0.67 (45.1-53.2)| 0.01±0.00 (nd)| 0.09±0.00 (nd-0.2)|
| C14:0      | 18.45±0.47 (16.8-21.0)| 0.01±0.00 (nd-0.3)| 10.9±0.08 (8.0-13.5)|
| C16:0      | 8.99±0.37 (7.5-10.2)| 7.97±0.04 (5.5-11.0)| 10.0±0.00 (nd-0.2)|
| C16:1      | 0.02±0.00 (nd)    | 0.03±0.00 (nd-1.2)| 10.0±0.00 (nd-0.2)|
| C18:0      | 3.19±0.23 (2.0-4.0)| 3.45±0.18 (3.0-6.5)| 4.81±0.16 (2.0-5.4)|
| C18:1      | 6.23±0.28 (5.0-10.0)| 22.40±0.10 (12.0-20.0)| 21.6±0.76 (17-30)|
| C18:2      | 1.37±0.05 (1.0-2.5)| 63.4±0.08 (58.0-78.0)| 52.9±0.70 (48.0-59.0)|
| C18:3      | 0.10±0.00 (nd-0.2) | 0.81±0.06 (nd-1.0) | 6.72±0.05 (4.5-11.0)|
| C20:0      | 0.01±0.00 (na)    | 0.23±0.01 (nd-1.0) | 0.35±0.01 (0.1-0.6)|
| C20:1      | nd                | 0.41±0.01 (nd-0.3) | 0.39±0.02 (nd-0.5)|

Values in parentheses are taken from reference values in Codex Alimentarius (2011). nd = not detected; na = not available

### RESULTS AND DISCUSSION

Table 1 listed the composition of fatty acid (FA) contained in VCO, grapeseed oil (GSO) and soybean oil (SO), as determined using gas chromatography with flame ionization detector, as recommended in several standard methods such as Codex Alimentarius and the American Oil Chemists’ Society (AOCS). Quantitative analysis of FAs was carried out using the normalization area, i.e., peak area of specific fatty acids was divided by the total peak area of all FAs in VCO, GSO and SO. FA profiles of VCO, GSO and SO were in agreement in the ranges appear in the standard of Codex Alimentarius commission (18). Therefore, it can be deduced that VCO, GSO and SO are not adulterated or mixed with other oils and are suitable for authentication study of VCO. The main FA composed VCO was lauric acid (C12:0), one of medium fatty acid, believed to be responsible in health-beneficial activities. The addition of SO and GSO, in turn, would reduce the levels of lauric acid. This reduction level could be used as an indicative that VCO has been mixed with other oils.

### Quantitative analysis

Fig. 1 showed FTIR spectra of VCO, GSO and SO at mid-infrared region (4000-650 cm-1). Each bands/peaks and shoulders are characteristics for FTIR spectra of triglyceride (TG). This is not surprising because the main components composed of edible fats and oils are TG. There are some bands and shoulders difference between VCO and two other oils, mainly at wavenumbers of about 2800 cm-1 and 1654 cm-1. Bands at wavenumbers of 2007 and 1654 cm-1 were absent in FTIR spectrum of VCO. These bands, corresponding to stretching vibration of unsaturation degree (=CH vinyl and C= C), were observed in FTIR spectra of GSO and SO. Based on fatty acid composition, GSO and SO contained much more unsaturated fatty acids than VCO, therefore it was not surprising if VCO did not reveal bands at 3007 and 1654 cm-1. The difference was also observed at wavenumbers of 1200-1095 cm-1, corresponding to ether (C-O) vibration. VCO showed one peak at 1117 cm-1, while GSO and SO revealed two peaks at 1117 and 1097 cm-1, respectively. These differences were used as the basis for classification and quantification of GSO and SO in VCO.
In order to quantify the levels of GSO and SO as oil adulterants model in VCO, the performance of two multivariate calibrations of partial least square (PLS) and principal component regression (PCR) were compared. FTIR spectra were also treated with Savitzky-Golay derivatization (first and second derivatives) for making the comparison of analytical results obtained using normal and derivative spectra. Derivatization would improve the resolution of the overlapping peak, but it would decrease the sensitivity. The selection of multivariate calibration and spectra types were relied on some statistical parameters, namely coefficient of determination ($R^2$), the number of factor, root mean square error of calibration (RMSEC), and root mean square error of prediction (RMSEP). The higher $R^2$ and the lower number of factors, RMSEC and RMSEP were preferred for quantification of GSO and SO in VCO.

Table 2 compiled the performance of PLS and PCR for quantification modelling of GSO and SO in VCO. Based on table 2, FTIR at normal spectra at the combined wavenumbers of 1200-900 and 3027-2985 cm$^{-1}$ assisted with PLS are chosen for quantification of GSO in VCO. PLS regression using FTIR normal spectra at combined
wavenumbers of 1200-1000 and 3025-2995 cm\(^{-1}\) was suitable for quantification of GSO in VCO. Fig. 2 revealed the correlation between actual values of GSO (x-axis) and FTIR predicted values (y-axis), either in calibration or validation models. The \(R^2\) close to 1 indicated that calibration and validation models were accurate, while the low values of RMSEC and RMSEP indicated good precision. Similarly, the presence of SO in VCO was quantified using PLS calibration model using the combined spectral region of 1200-1000 and 3025-2995 cm\(^{-1}\). This wavenumbers region revealed spectral difference between SO and VCO, thus making PLS model for the relationship between actual value and FTIR predicted values, as indicated by high \(R^2\) value and low RMSEC and RMSEP values.

### Table 2: The performance of multivariate calibration of partial least square (PLS) and principle component regression (PCR) for quantitative analysis of grapeseed oil and soybean oil as adulterants in virgin coconut oil (VCO)

| Adulterants, frequency regions selected | Multivariate calibration | Spectra Factor | Equation Calibration | Validation | \(R^2\) Calibration | \(R^2\) Prediction | RMSEC (%) | RMSEP (%) |
|----------------------------------------|-------------------------|----------------|----------------------|------------|---------------------|---------------------|-----------|-----------|
| Grape seed oil, 1200-900                | PLS                     | Normal 9       | \(y = 0.997x+0.035\) | \(y = 1.038x-1.056\) | 0.998 | 0.994 | 0.007 | 1.32 |
|                                        |                         | 1st der 5      | \(y = 0.999x+0.075\) | \(y = 0.907x+2.040\) | 0.994 | 0.975 | 1.17 | 2.48 |
|                                        |                         | 2nd der 10     | \(y = 0.966x+0.433\) | \(y = 0.404x+9.876\) | 0.991 | 0.409 | 0.091 | 11.9 |
|                                        | PCR                     | Normal 10      | \(y = 0.993x+0.098\) | \(y = 0.873x+4.104\) | 0.998 | 0.981 | 0.622 | 2.80 |
| Soybean oil, 1200-1000                  | PLS                     | Normal 5       | \(y = 1.011x+0.196\) | \(y = 1.042x-1.098\) | 0.999 | 0.996 | 0.268 | 1.04 |
|                                        |                         | 1st der 5      | \(y = 0.999x+0.011\) | \(y = 0.939x+1.644\) | 0.999 | 0.989 | 0.334 | 1.70 |
|                                        |                         | 2nd der 6      | \(y = 0.998x+0.028\) | \(y = 0.762x+3.380\) | 0.998 | 0.877 | 0.532 | 5.53 |
|                                        | PCR                     | Normal 10      | \(y = 0.999x+0.003\) | \(y = 0.990x-0.187\) | 0.999 | 0.995 | 0.208 | 1.05 |
|                                        |                         | 1st der 10     | \(y = 0.999x+0.010\) | \(y = 0.939x+1.489\) | 0.999 | 0.990 | 0.337 | 1.64 |
|                                        |                         | 2nd der 10     | \(y = 0.992x+0.151\) | \(y = 0.780x+3.579\) | 0.992 | 0.915 | 1.22 | 4.81 |

* Spectral treatments and multivariate calibrations chosen for analysis of adulterants are italicized. PLS = partial least square; PCR = principle component regression; RMSEC = root mean square error of calibration; RMSEP = root mean square error of prediction.

**Discriminant analysis**

The chemometrics of discriminant analysis (DA), one of supervised pattern recognition techniques, was used for making classification between VCO and VCO adulterated with GSO and SO. The wavenumbers region used for quantitative analysis were used for classification. The Coomans plots for the classification of VCO adulterated with GSO and SO was shown in fig. 3. DA can classify pure VCO and that adulterated with GSO and SO with an accuracy level of 100%.
CONCLUSION

FTIR spectroscopy combined with multivariate calibrations of PLS and PCR and discriminant analysis (DA) has been developed for adulteration analysis of VCO with GSO and SO. FTIR normal spectra combined with PLS is successfully used for quantification of GSO and SO with acceptable accuracy and precision. In addition, DA can classify VCO and VCO adulterated with GSO and SO accurately.

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AUTHORS CONTRIBUTIONS

AR performed research activities and prepared manuscript. YBCM and MEA designed research and made critical thinking on the manuscript.

CONFLICTS OF INTERESTS

All authors have none to declare

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