

# DETECTION AND QUANTIFICATION OF SOYBEAN AND CORN OILS AS ADULTERANTS IN AVOCADO OIL USING FOURIER TRANSFORM MID INFRARED (FT-MIR) SPECTROSCOPY AIDED WITH MULTIVARIATE CALIBRATION

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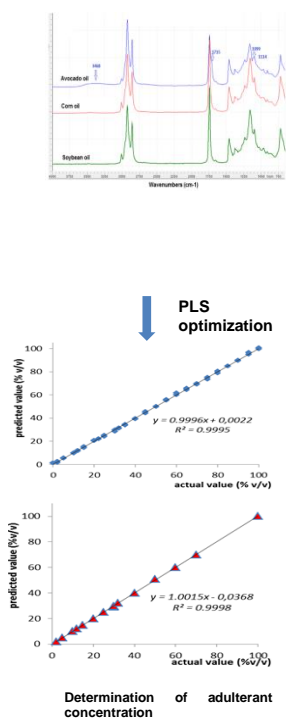
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## Graphical abstract



## Abstract

Authentication of high value edible oils like avocado oil (AO) is an emerging issue. AO can be target of adulteration with low priced oils like soybean and corn oils. The present study is intended to quantify soybean oil (SO) and corn oil (CO) in avocado oil (AO) using the combination of Fourier transform mid infrared (FT-MIR) spectroscopy and chemometrics. The quantification was carried out by partial least square (PLS) calibration using some spectral processing, namely normal spectra, smoothing, and derivation treatment. Frequencies of 1427-779 cm<sup>-1</sup> with normal spectra were suitable for the quantification of SO in AO which revealed high coefficient determination (R<sup>2</sup>), i.e. 0.9994 and low root mean square error of calibration (RMSEC), i.e. 0.86% (v/v). Meanwhile, R<sup>2</sup> of 0.9994 and RMSEC of 0.87% (v/v) were obtained by PLS at the combined spectra at frequency regions of 1477-721, 1728-1685, and 3035-2881 cm<sup>-1</sup> for quantification of CO in AO. The model was further validated using independent samples and offered high R<sup>2</sup> values of 0.9994 (for CO) and 0.9998 (for SO) with root mean square error of prediction (RMSEP) of 0.88% (v/v) (CO) and 0.52 % (v/v) (SO), respectively. In general, FT-MIR spectroscopy serves rapid and accurate determination of CO and SO in AO for authenticity studies.

Keywords: FT-MIR spectroscopy, avocado oil, chemometrics, partial least square

## Abstrak

Pengesahan minyak makan mahal seperti minyak alpukat (AO) merupakan isu yang baru muncul. AO boleh menjadi sasaran pencemaran dengan minyak murah seperti minyak kacang soya dan jagung. Kajian ini bertujuan untuk mengukur minyak kacang soya (SO) dan minyak jagung (CO) dalam minyak alpukat (AO) menggunakan gabungan spektroskopi FT-MIR dan kemometrik. Hitungan PLS menggunakan pemrosesan spektrum, iaitu spektrum normal, smoothing, dan rawatan turunan. Kekekapan 1427-779 cm<sup>-1</sup> pada spektrum normal sesuai untuk kuantifikasi SO yang mendedahkan penentuan R<sup>2</sup>, iaitu 0.9994 dan root mean square of error calibration (RMSEC), iaitu 0.86% (v/v). Sementara itu, R<sup>2</sup> daripada 0.9994 dan RMSEC sebanyak 0.87% (v/v) diperolehi oleh PLS pada

spektrum gabungan di kawasan kekerapan 1477-721, 1728-1685 dan 3035-2881  $\text{cm}^{-1}$  untuk kuantifikasi CO di AO. Model disahkan menggunakan sampel bebas dan menawarkan nilai  $R^2$  yang tinggi 0,9994 (untuk CO) dan 0,9998 (untuk SO) dengan root mean square of prediction (RMSEP) sebanyak 0,88% (v/v) (CO) dan 0,52% (v/v) (SO). Secara umum, FT-MIR spektroskopi berfaedah dalam penentuan cepat dan tepat CO dan AO untuk kajian ketulenan.

*Kata kunci:* spektroskopi FT-MIR, minyak alpukat, kemometrik, partial least square

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## 1.0 INTRODUCTION

Avocado belongs to Lauraceae family. It is also known as aguacate or avocadobirne. In Indonesia, Avocado is called as Alpukat. It is a native plant from Central America such as Guatemala and Honduras. Currently, this plant has spread to all parts of the world. It becomes popular fruit due to the pleasant flavor and good taste. Besides, it contains many vitamins and other nutritious elements which are beneficial for the human health [1, 2]. The content of oils in avocado pulp is high, depending on the cultivar and the growth conditions [3]. Avocado oil (AO) is suggested to be a part of our daily diet, especially for the prevention of cardiovascular disease, since it can lower the level of low density lipoprotein (LDL) and triglyceride in blood [4]. According to Requejo [5], AO is also known as oil having high content of unsaturated fatty acids, phytosterol, and antioxidant agents similar to olive oil.

The authentication of AO becomes very important, since the price of AO in the market of fats and oils is several times from that of common vegetable oils such as palm oil, corn oil and soybean oil. Avocado oil can be adulterated with these vegetable oils. The authenticity of expensive vegetable oils is an interesting issue since it is associated with health and economic aspect of consumers. Besides, the authenticity can be also related to the prohibition of certain religion like lard adulteration in vegetable oil [6]. Therefore, the detection and quantification of oil adulterants in AO must be addressed to assure its quality and safety.

Several analytical methods have been developed for the detection and quantification of adulterants in edible fats and oils such as differential scanning calorimetry (DSC) [7]; chromatographic method [8]; nuclear magnetic resonance spectroscopy (NMR) [9]; and wet chemical methods [10]. However, some of these methods are time consuming, expensive, and generally destructive of the sample material. Therefore, rapid and accurate analytical methods must be developed for detecting and quantifying the oil adulterants. Such method offering reliable and rapid analytical result is Fourier transform infrared (FTIR) spectroscopy. Vibrational spectroscopy techniques such as Raman spectroscopy [11] and mid-infrared spectroscopy [12] have been reported to authenticate high price edible oils.

FTIR spectroscopy has received great attention in the quantitative analysis of fats and oils over the years due to the simplicity with reduced or no-sample pre-treatment steps. This technique can be considered as "green analytical chemistry" because the use of solvent and chemical reagents are eliminated [13]. FTIR spectroscopy combined with some chemometrics techniques has been successfully used to authenticate black cumin oil in ternary mixture with corn and soybean oil [14], black cumin in binary mixture with grapeseed oil, extra virgin oil adulterated with palm oil [15], and virgin coconut oil in binary mixture with sunflower and corn oil [16]. However, there is no report related to the authentication of AO using combination of FTIR spectroscopy and chemometrics. Therefore, in this research, we develop rapid quantification of corn oil (CO) and soybean oil (SO) in AO by combining the FT-MIR spectroscopy and chemometrics technique

## 2.0 EXPERIMENTAL

Avocado oil was obtained by extracting avocado pulp by cold percolation technique using n-hexane as the solvent. The used avocado fruit is the mixture avocado from three different regions in Yogyakarta and Central Java. Corn oil and soybean oil were purchased from the local market in Yogyakarta, Indonesia. These oils were packaged in polyethylenephtalate (PET) bottles until being used for analysis. For FTIR spectra analysis, the calibration and validation samples composed of binary mixtures of AO-SO and AO-CO were prepared separately in the concentration ranges of 2.0-95.0% (v/v). The mixtures were shaken vigorously to ensure the total homogenization.

### 2.1 Measurement of FTIR Spectra

The FTIR spectra of samples were obtained using ABB 3000 FTIR-ATR spectrophotometer (Canada) with attenuated total reflectance (ATR) crystal of ZnSe equipped with deuterated triglycine sulphate (DTGS) as detector, and potassium bromide (KBr) as beam splitter. The instrument was integrated with the HorizonMB 3000 software for FTIR spectra processing. The measurements were directly carried out by

placing oil samples on ATR surface at controlled room temperature (25°C) in MIR region (4000–650  $\text{cm}^{-1}$ ). These spectra were subtracted from reference spectrum of air, acquired by collecting a spectrum from the cleaned blank ATR crystal before the measurement of each oil sample replication. The sample spectra were collected in duplicate and displayed as the average spectra. At the end of every scan, the crystal was cleaned with hexane twice and dried with special soft tissue, cleaned with acetone, and finally dried again with soft tissue following the collection of each spectrum.

## 2.2 Chemometrics Analysis

The Data analysis using PLS was performed using the Horizon MB™ FTIR software. The frequency regions were automatically selected by the software and were confirmed by investigating peaks where the variations were observed. The values of root mean square error of calibration (RMSEC) and coefficient of determination ( $R^2$ ) were used as validity criteria for PLS calibration model. The optimum number of PLS was determined by cross-validation. The predictability of models was verified by computing root mean square of prediction (RMSEP) and coefficient of determination.

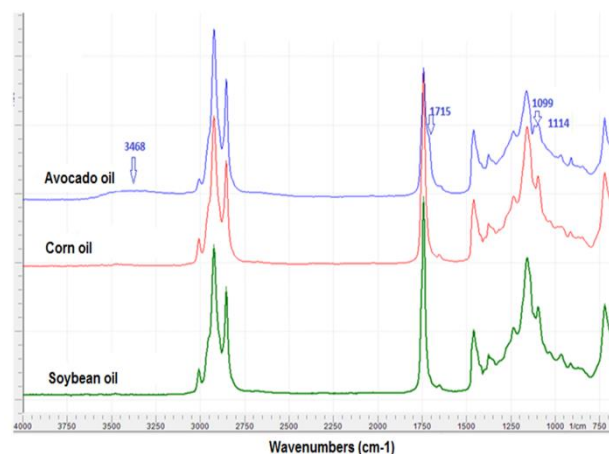
## 3.0 RESULTS AND DISCUSSION

### 3.1 FT-MIR Spectral Analysis

Figure 1 showed FTIR spectra of authentic AO, SO, and CO at regions of 4000–650  $\text{cm}^{-1}$ . Each band and shoulder in FTIR spectra corresponds to the functional group responsible for IR absorption and exhibits the characteristic bands for edible fats and oils. The entire spectra look very similar and are dominated by FTIR spectra triglycerides. The main components of edible fats and oils are triglycerides (nearly 98%). The other components of edible fats and oils are sterols, vitamins, diacylglycerols (DAGs), monoacylglycerols and free fatty acids. The However, if one examines the spectra closely, they reveal some differences in terms of peak intensities which can be observed in the region around 1750–1700  $\text{cm}^{-1}$  as well as at the fingerprint region, especially in 1085–1200 and 3468  $\text{cm}^{-1}$ . Due to the fingerprint technique meaning that two different oils will reveal different FTIR spectra, FTIR spectroscopy can be used to extract the differences among these oils [17].

No bands were observed for SO and CO at 1715  $\text{cm}^{-1}$ , while AO has absorption bands at 1715  $\text{cm}^{-1}$  due to C=O stretching vibration. The stretching vibration of -OH from  $\beta$ -cytosterol at 3468  $\text{cm}^{-1}$  in AO spectra was not present in SO and CO. Furthermore, AO revealed 2 peaks at 1099 and 1114  $\text{cm}^{-1}$ . Both originated from C-O stretching vibration [18, 19]. The differences of fatty acids composition of those oils contributed to the different peak intensities revealed, especially at frequency of 975–900  $\text{cm}^{-1}$ . These frequency regions

can be exploited for the quantification of either SO or CO in the binary mixtures with AO.

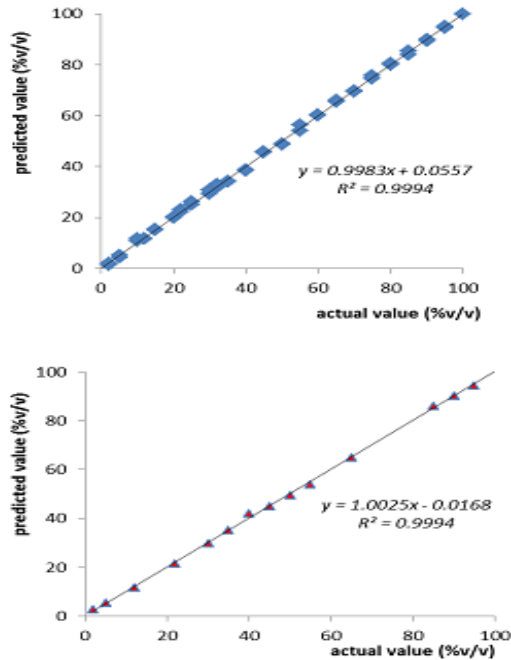


**Figure 1** FTIR spectra of avocado oil, soybean oil and corn oil scanned in mid-infrared region (4000–400  $\text{cm}^{-1}$ )

### 3.2 Quantitative Analysis of SO in Binary Mixture with AO

After optimizing the frequency regions which are prefer for the quantification of SO, the frequency region of 779–1477  $\text{cm}^{-1}$  was finally chosen. At this region, the highest value of  $R^2$  (0.9994) and the lowest value of RMSEC (0.86% (v/v)) were achieved. This  $R^2$  value informed that the predictors are able to describe 99.94% variation contained in the dependent variables. The low value of RMSEC suggests the ability of calibration model to explain the relationship between predictor and responses with low random errors. The lower RMSEC, the better model was obtained [16]. The risk of multivariate analysis use is overfitting phenomenon due to the noise contributing during modelling. Overfitting occurs when the calibration model offers good  $R^2$  and low RMSEC in calibration models, but the model shows bad performance in predicting the unknown samples. Further, the validation (either internal using leave one out technique or external using independent samples) is required for solving the problem. In this study, we choose external validation by preparing some independent samples which are different from calibration samples [20].

The relationship between actual value of SO and FTIR predicted values during validation was depicted in Figure 2 with  $R^2$  0.9998. The RMSEP value obtained is 0.35%, which is close to the RMSEC value obtained. The closeness between RMSEC and RMSEP indicate the accuracy and robustness of the model [21]. In addition, the model has the good performance to predict the concentration of independent samples.



**Figure 2** Relationship between actual value and FTIR predicted value of soybean oil (◆: calibration, ▲: validation)

### 3.3. Quantitative Analysis of Corn Oil (CO) in Binary Mixture with AO

For CO quantification, the normal spectra at the combined frequency region of 3035-2881, 1728-1685, and 1477-721  $\text{cm}^{-1}$  were selected. PLS calibration at this combination region revealed the highest  $R^2$  of 0.9994 as shown in Figure 3 with the lowest RMSEC of 0.87% (v/v). In order to avoid the possibility of overfitting in the obtained model, cross-validation using leave-one out technique was employed. Three factors was included in constructing the quantification model based on the minimum PRESS value. Besides, the model was further validated with independent samples to verify its predictive capability. The value of coefficient determination was 0.9994 with RMSEP value of 0.88% (v/v).

The result showed that the PLS model can predict the level of CO accurately with no overfitting observed, since the RMSEC and RMSEP value was close to each other. The performance of PLS for quantification was compiled in Table 1.

**Table 1** PLS performances for determination of SO and CO in binary mixture with avocado oil (AO)

Frequency regions ( $\text{cm}^{-1}$ )	adulterants	spectra	PLS factor	$R^2$		RMSEC
				Calibration	Validation	
1427-779	soybean oil	normal	6	0.9995	0.9998	0.85787
		1 <sup>st</sup> derivative	4	0.9984	0.9995	1.09772
		2 <sup>nd</sup> derivative	4	0.9980	0.9993	1.16238
1477-721; 1728-1685; and 3035-2881	corn oil	normal	6	0.9994	0.9994	0.8697
		1 <sup>st</sup> derivative	5	0.9994	0.9993	0.8761
		2 <sup>nd</sup> derivative	5	0.9993	0.9993	0.8940

## 4.0 CONCLUSION

It can be stated that FT-MIR spectroscopy can be effective mean for quantifying the level of avocado oil (AO) adulterants, namely soybean oil (SO) and corn oil (CO). The level of SO and CO is quantified using PLS calibration at frequency region of 779-1477  $\text{cm}^{-1}$  (for SO) and at the combined frequency region of 3035-2881, 1728-1685, and 1477-721  $\text{cm}^{-1}$  (for CO). The  $R^2$ , RMSEC, and RMSEP values obtained indicated that the developed model can quantify SO and CO accurately and precisely. FTIR spectroscopy offers simple, rapid and no excessive sample preparation technique for authentication study of edible oils.

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## References

- [1] V. Ashworth, H. Chen and M. T. Clegg. 2011. *Persea*, in: Wild Crop Relatives: Genomic and Breeding Resources, Tropical and Subtropical Fruits, C.Kole ed. Berlin: Springer-Verlag.
- [2] G. Flores, M. L. Ruiz Del Castillo, G. P. Blanch, and M. Herraiz. 2007. Detection of the Adulteration of Olive Oils by Solid Phase Microextraction and Multidimensional Gas Chromatography. *Food Chemistry*. 97: 336-342.
- [3] F. Takenaga, K. Matsuyama, S. Abe, Y. Torri and S. Itoh. 2008. Lipid and Fatty Acid Composition of Mesocarp and Seed of Avocado Fruits Harvested at Northern Range in Japan. *Journal of Oleo Science*. 57: 591-597.
- [4] A. Ortiz-Moreno, L. Dorantes, J. Galindez and R. I. Guzman. 2003. Effect of Different Extraction Methods on Fatty Acids, Volatile Compounds, and Physical and Chemical Properties of Avocado (*Persea Americana* Mill.) Oil. *Journal of Agricultural and Food Chemistry*. 51: 2216-2221.
- [5] A. M. Requejo, R. M. Ortega, F. Robles, B. Navia, M. Faci and A. Aparicio. 2003. Influence of Nutrition on Cognitive Function in a Group of Elderly, Independently Living People. *European Journal of Clinical Nutrition*. 57: S54-S57.

- [6] A. Rohman, and Y. B. Che Man. 2012. Application of Fourier Transform Infrared Spectroscopy for Authentication of Functional Food Oils. *Applied Spectroscopy Reviews*. 47(1): 1-13.
- [7] E. Chiavaro, E. Vittadini, M. T. Rodriguez-Estrada, L. Cerretani, L. Capelli, and A. Bendini. 2009. Differential Scanning Calorimetry Detection of High Oleic Sunflower Oil as an Adulterant in Extra-Virgin Olive Oil. *Journal of Food Lipids*. 16: 227-244.
- [8] R. Aparicio, R. Aparicio-Ruiz, and D. L. Garzia-Gonzalez. 2007. Rapid Methods for Testing of Oil Authenticity: The Case of Olive Oil. In *Rapid Methods for Food & Feed Quality Determination*, ed. A. van Amerongen, D., Barug, and M. Lauwaars, Wageningen Academic Publisher, The Netherlands. 117-118.
- [9] M. Jafari, M. Kadivar and J. Keramat. 2009. Detection of Adulteration in Iranian Olive Oils Using Instrumental (GC, NMR, DSC) Methods. *Journal of the American Oil Chemists' Society*. 86: 103-110.
- [10] A. Rohman and Y. B. Che Man. 2009. Monitoring of Virgin Coconut Oil (VCO) Adulteration with Palm Oil Using Fourier Transform Infrared (FTIR) Spectroscopy. *Journal Food Lipids*. 16: 618-628
- [11] H. M. Heise, U. Damm, P. Lampen, A. N. Davies, and P. S. McIntyre. 2005. Spectral Variable Selection For Partial Least Squares Calibration Applied to Authentication and Quantification of Extra Virgin Olive Oils Using Fourier Transform Raman Spectroscopy. *Applied Spectroscopy*. 59: 1286-1294.
- [12] G. Gurdeniz and B. Ozen. 2007. Detection of Adulteration of Extra-Virgin Olive Oil by Chemometric Analysis of Mid-Infrared Spectral Data. *Food Chemistry*. 116: 519-525.
- [13] A. F. Nurrulhidayah, Y. B. Che Man, H. A. Al-Kahtani and A. Rohman. 2011. Application of FTIR Spectroscopy Coupled with Chemometrics for Authentication of Nigella Sativa Seed Oil. *Spectroscopy*. 25: 243-50.
- [14] A. Rohman, and R. Ariani. 2013. Authentication of Nigella Sativa Seed Oil in Binary and Ternary Mixtures with Corn Oil and Soybean Oil using FTIR Spectroscopy Coupled with Partial Least Square. *TSWJ-Analytical Chemistry*. Volume 2013, Article ID 740142, 6 pages <http://dx.doi.org/10.1155/2013/740142>.
- [15] A. Rohman and Y.B. Che Man. 2012. Fourier Transform Infrared (FTIR) Spectroscopy for Analysis of Extra Virgin Olive Oil Adulterated with Palm Oil. *Food Research International*. 43: 886-892.
- [16] A. Rohman and Y. B. Che Man. 2011. The Use of Fourier Transform Mid Infrared (Ft-Mir) Spectroscopy for Detection and Quantification of Adulteration in Virgin Coconut Oil. *Food Chemistry*. 129(2): 583-588.
- [17] A. Rohmana, I. Gupitasari, Purwantoa, K. Triyanaa, A. S. Rosman, S. A. S. Ahmad, and F. Mohd Yusof. 2013. Quantification of Lard in the Mixture with Olive Oil in Cream Cosmetics Based on FTIR Spectra and Chemometrics for Halal Authentication. *Jurnal Teknologi*. 69: 113-119.
- [18] Lerma-Garcia, M. J., Ramis-Ramos, G., Herrero-Martinez, J. M., Simo-Alfonso, E. F. 2010. Authentication of Extra Virgin Olive Oils by Fourier Transform Infrared Spectroscopy. *Food Chemistry*. 118: 78-83.
- [19] E. Lukitaningsih, M. Saadah, Purwanto, and A. Rohman. 2012. Quantitative Analysis of Lard in Lotion Cosmetics Formulation Using FTIR Spectroscopy and Partial Least Square Calibration. *Journal of the American Oil Chemists Society*. 89: 1537-1543.
- [20] Miller, J. N., and Miller, J. C. 2010. *Statistics and Chemometrics for Analytical Chemistry*. The 6<sup>th</sup> Ed. Edinburgh Gate Harlow Essex, England: Pearson Education Limited. 221-250.
- [21] Rohman, A., Che Man, Y. B., & Riyanto, S. 2011. Authentication Analysis of Red Fruit (*Pandanus conoideus* Lam) Oil Using FTIR Spectroscopy in Combination with Chemometrics. *Phytochemical Analysis*. 22(5): 462-467.