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Application News

No. AD-0167

Halal Authentication Analysis / IRTracer-100

Quantitative Determination of Lard Adulteration by FTIR Spectroscopy with Chemometrics Method - Virgin Coconut Oil

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❑ Introduction

In recent years there has been a considerable amount of interest in virgin coconut oil (VCO) due to its potential health benefits [1]. Moreover, VCO has a higher commercial value compared to other edible oils. This has led to VCO to be a target for potential adulteration with cheaper oil or animal fat such as lard. Lard resembles VCO due to its creamy white and solid form appearance at room temperature. Such adulteration practice is a commercial fraud which could have a negative economic implication. Therefore, there is a need to establish a reliable method to detect and quantitate lard adulteration in VCO.

Fourier Transform Infrared (FTIR) spectroscopy is a useful technique in identification of organic component due to its characteristic absorption in the infrared (IR) region. Furthermore, when being used in conjunction with chemometrics data analysis such as partial least squares (PLS) regression, it enables a rapid quantitative analysis with minimal sample preparation. This application news examines two measurement and quantitation methods, attenuated total reflection (ATR) with PLS quantitation and transmission with calibration curve quantitation, for the determination of lard adulterated VCO using FTIR spectroscopy.

❑ Experimental

Pork lard and commercially available VCO were purchased from local markets. The lard was extracted based on the procedure by Rohman and Che Man [2]. A set of 17 standards containing 0.5 – 30% (w/w) lard in VCO was prepared. This set of standards was measured using two different methods – ATR and transmission method.

In FTIR-ATR analysis, the sample was measured with horizontal type ATR attachment with zinc selenide (ZnSe) prism. Each sample was measured three times. The IR spectra were acquired in the wavenumber range of 4000 cm^{-1} – 650 cm^{-1}. The measurement conditions are shown in Table 1.

A PLS calibration model for lard was established with 15 of these standards using LabSolutions IR workstation with Chemometrics PLS function. The remaining 2 standards were used as samples for quantitative determination.

Table 1: Instruments and analytical conditions

Instruments	: IRTracer-100, ATR-8200H (ZnSe)
Resolution	: 4.0 cm^{-1}
Accumulation	: 45
Apodization	: Happ-Genzel
Detector	: DLATGS

In FTIR transmission analysis, Specac Pearl™ liquid transmission accessory, as shown in Figure 1, with a path length of 100 µm was used. A drop of the sample was added to the bottom ZnSe window, and the droplet was then sandwiched with the top window. This process is easier and faster than using traditional liquid fixed thickness cell. Each standard was measured once and the IR spectra was measured ranging from 4000 cm^{-1} – 650 cm^{-1}. The measurement conditions are shown in Table 2.

Table 2: Instruments and analytical conditions

Instruments	: IRTracer-100, Pearl™ (ZnSe, 100 µm)
Resolution	: 4.0 cm^{-1}
Accumulation	: 45
Apodization	: Happ-Genzel
Detector	: DLATGS

Figure 1: Specac Pearl™ liquid transmission accessory

❑ Results and Discussion

FTIR-ATR with PLS Quantitation Method

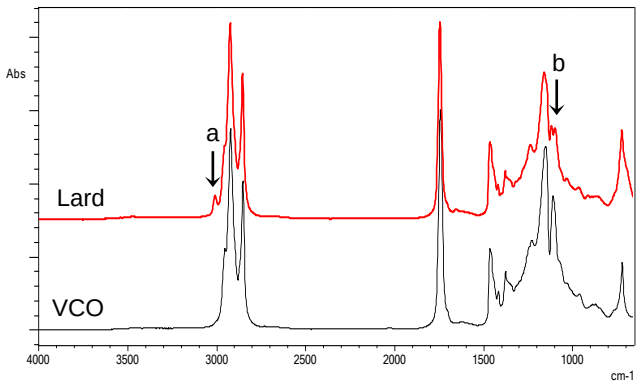

Figure 2: IR spectra of VCO and lard

Figure 2 shows the IR spectra of VCO and lard. The IR spectra of VCO and lard were quite similar, however there are some differences in the IR regions as indicated by arrows in Figure 2. A peak is present at around 3007 cm^{-1} for lard as shown by arrow (a) and is not observed for VCO. Peak at 3007 cm^{-1} is attributed to *cis* –C=H vibration and correlated with the presence of unsaturated fatty acid [3]. Coconut oil contains more than 90% of saturated fatty acid. Hence, a presence of peak at 3007 cm^{-1} may indicate an adulteration in coconut oil. In addition, at around IR range of 1120 – 1090 cm^{-1}, lard has two peaks at around 1097cm^{-1} and 1117 cm^{-1}, whereas VCO has only one peak at around 1110 cm^{-1} as shown by arrow (b). Figure 3 shows the IR spectra of VCO and lard in the IR range of 1220 – 1050 cm^{-1}.

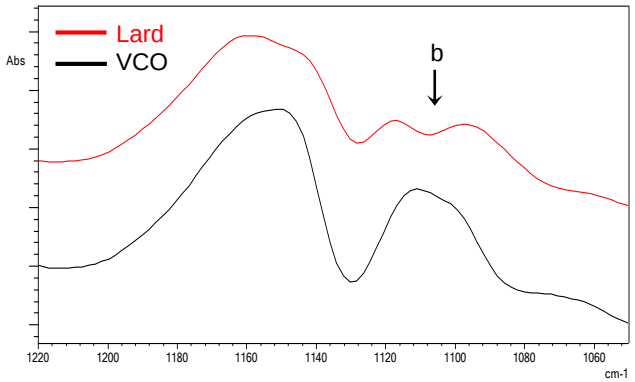

Figure 3: IR spectra of VCO and lard in IR range 1220 – 1050 cm^{-1}

A PLS calibration model was created using calibration standards of lard in VCO with concentration ranging from 0.5 – 30% w/w. Second derivative spectra were used in the PLS data analysis for better resolution of overlapping and shoulder peaks. Table 3 and Figure 4 show the PLS calibration parameter and result of lard in VCO.

Table 3: PLS calibration parameters of lard in VCO

Calibration Table	
Algorithm	PLS I
Number of references	51 (three measurement per sample)
Range (cm^{-1})	900 – 1500 2750 – 3050
Pre-process	Derivative, Order = 2, Points = 5
Scale	Autoscale
Number of factors	4
Square of correlation coefficient	0.9999
MSEP	0.0001
SEP	0.0098

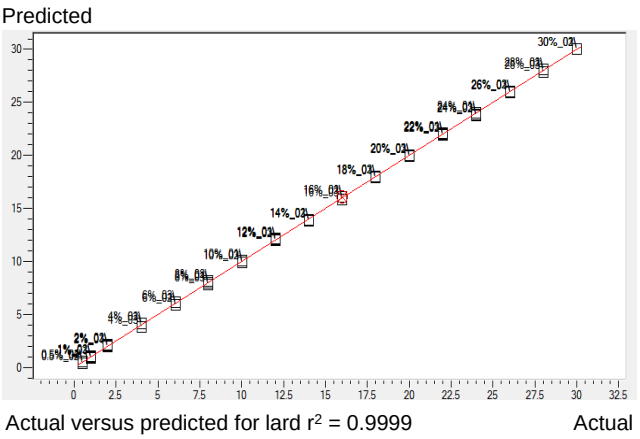

Figure 4: PLS calibration for lard in VCO predicted versus actual values

A good square of correlation coefficients of more than 0.999 was obtained for the PLS calibration modelling with low Mean Squared Error of Prediction (MSEP) and low Standard Error of Prediction (SEP).

Table 4 shows the quantitation results of lard in two different brands of VCO by PLS quantitation method. The measurement results are within ±10% of the expected values for lard in VCO.

Table 4: Predicted values of lard in two brands of VCO

Edible Oil		**Brand A VCO**		**Brand B VCO**	
Expected Value (% w/w)		2.5	5.0	2.5	5.0
Predicted Value (% w/w)	**1**	2.36	4.93	2.53	5.33
	2	2.44	5.31	2.62	5.29
	3	2.55	5.37	2.62	5.23
	Mean	2.45	5.20	2.59	5.28
Recovery (%)		98.0	104.1	103.6	105.7

Transmission with Calibration Curve Quantitation Method

A calibration curve was generated using 6 calibration standards of lard in VCO with concentration ranging from 1 – 10% w/w. The peak at around 3007 cm^{-1} from lard is selected for calibration. Figure 5 shows the IR spectra of lard in VCO calibration standards in the IR range of 3030 – 2992 cm^{-1}.

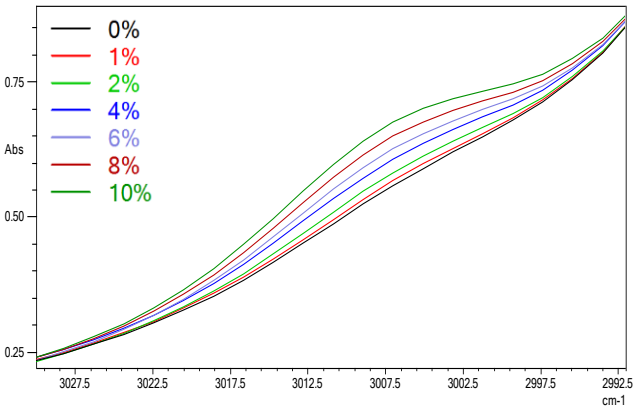

Figure 5: IR spectra of lard in VCO calibration standards in IR range 3030 – 2992 cm^{-1}

Table 5 shows the parameter setting for the generation of the calibration curve. A good square correlation coefficient of 0.997 was obtained. The calibration curve is shown in Figure 6.

Table 5: Calibration curve parameters of lard in VCO

Calibration Curve Parameters	
Component	Lard in VCO
Unit	%
Quantitation Method	Peak Area
Range (cm^{-1})	2992 – 3030, BC: ON
Calibration Curve	Multi-point
Order	1st order
Square of correlation coefficient	0.9973

Table 6 shows the quantitation results of lard in two different brands of VCO based on the transmission method. The measurement results are within ±10% of the expected values for lard in VCO.

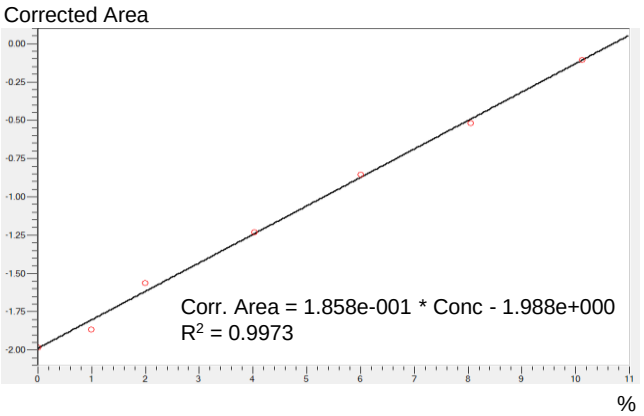

Figure 6: Calibration curve of lard in VCO

Table 6: Quantitation results of lard in two brands of VCO

Edible Oil		Brand A VCO		Brand B VCO	
Expected Value (% w/w)		2.5	5.0	2.5	5.0
Predicted Value (% w/w)	**1**	2.42	5.15	2.33	5.22
	2	2.52	5.20	2.31	5.24
	3	2.66	5.37	2.27	5.20
	Mean	2.53	5.24	2.30	5.22
Recovery (%)		101.3	104.8	92.1	104.4

❑ Conclusions

Using either FTIR-ATR with PLS quantitation method or transmission with calibration curve quantitation method, a good square correlation coefficient of more than 0.995 was obtained with a good recovery of within ±10% for the verification samples. This shows both methods have potential for quantitative determination of lard adulteration in VCO.

❑ References

1. Marina, A.M., Che Man, Y.B. and Amin, I. (2009). Virgin coconut oil: emerging functional food oil. Trends in Food Science and Technology, 20: 481-487.
2. Rohman, A. and Che Man, Y. B. (2009). Analysis of cod-liver oil adulteration using Fourier transform infrared (FTIR) spectroscopy. Journal of the American Oil Chemists' Society, 86, 1149–1153.
3. Rohman, A. and Che Man, Y. B. (2011). Simultaneous quantitative analysis of two functional food oils, extra virgin olive oil and virgin coconut oil using FTIR spectroscopy and multivariate calibration. International Food Research Journal 18(4): 1231-1235.

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