

## Fourier Transform Infrared Spectroscopy applied for rapid analysis of lard in palm oil

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**Abstract:** The presence of lard in vegetable oils like palm oil is serious matter for certain communities like Muslim and Hindis. Fourier transform infrared (FTIR) spectroscopy has been used for determination of lard in palm oil. Lard, palm oil and their binary mixtures in the neat form was scanned at mid infrared region (4,000 – 650  $\text{cm}^{-1}$ ) using attenuated total reflectance accessory as convenient sampling technique. The level of lard in palm oil was determined at frequency regions of 1480 – 1085  $\text{cm}^{-1}$  with the aid of partial least square. The relationship between actual value of lard (*x-axis*) and FTIR predicted value (*y-axis*) showed linear correlation with coefficient of determination of 0.998. The root mean square error of calibration (RMSEC) value of lard was 1.69 %, while the value of square error of cross validation was 2.87 % (v/v). This result indicated that FTIR spectroscopy is accurate and precise enough for such determination.

**Keywords:** FTIR spectroscopy, lard, palm oil, partial least square

### Introduction

Lard, one type of animal fats, is frequently used with other plant oils such as olive and palm oils to produce shortenings, margarines and other speciality food oils (Marikkar *et al.*, 2005). Lard can be taken into account from 2 points of view, economical and religion. In certain countries, the producers of food products prefer to blend vegetable oils with lard in order reduce the production cost (Rohman and Che Man, 2010). Any food products containing lard is not allowed due to religious reason. Some religions like Islam, Judaism and Hinduism not allowed their followers to consume any foods containing lard in its formulation (Regenstein *et al.*, 2003); consequently, analytical method for detecting lard is highly needed.

Indonesia and Malaysia is the largest exporting countries of palm oil (Gunston, 2011). Palm oil is one of the major oils consumed not only in Asian region regions, but also in the worldwide. Palm oil consists more than 90% triacylglycerols, 2–7% diacylglycerols, 0.1 % monoacylglycerols, 3–5 % free fatty acids and 1% minor components. The minor components are a complex mixture of carotenoids, tocopherols (tocopherols and tocotrienols), squalene, sterols, and phospholipids (Puah *et al.*, 2007). For

reducing the production cost, lard was sometimes added to palm oil to produce specialty food oils such as butter and margarine. Therefore, the detection of lard in palm oil is necessary to assure the halalness of food containing lard.

Analysis of edible fats and oils is usually done by determining specific components such as fatty acids, triacylglycerols, and tocopherols rather than analysis of lard as part of the whole food systems (Rohman and Che Man, 2012). Fourier transform infrared (FTIR) spectroscopy offers analysis of sample as a whole matter. This technique is capable of determining analyte(s) of interest, especially in combination with chemometrics techniques of multivariate calibration (partial least square, PLS). Besides, FTIR spectroscopy in combination of attenuated total reflectance is not involving excessive sample preparation and not exploiting the use of chemicals and reagents; therefore, FTIR spectroscopy for analysis of edible fats and oils can be considered as “green analytical method” (Namies’nik, 2001).

FTIR spectroscopy in combination with partial least square has been used for determination of lard in selected vegetable oils (Rohman *et al.* 2011); lard in the mixture with other animal fats namely chicken, beef and mutton fats (Rohman and Che Man, 2010), and lard in cod liver oil (Rohman and

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Che Man, 2009). We also determined lard present in cream cosmetics (Rohman and Che Man, 2011). Currently, Che Man *et al.* (2011) also differentiate lard from other animal fats and other vegetable oils using FTIR spectroscopy in combination with principal component analysis (PCA). However, there is no reported data regarding the application of FTIR spectroscopy for determination of lard in palm oil. In this study, FTIR spectroscopy with the accessory of horizontal attenuated total reflectance (HATR) in conjunction with chemometrics techniques of partial least square (PLS) was used for quantification of lard in palm oil.

## Materials and Methods

### *Lard preparation*

Lard was prepared by rendering adipose tissue of pig according to previously reported procedure by Rohman and Che Man (2009). Adipose tissue of pig was purchased from various slaughtered house around Yogyakarta, Indonesia. In this process, the tissues were cut into small pieces, mixed, and melted at 90–100°C for 2 h in the oven. The melted fat was strained through triple-folded muslin cloth, dried by addition of anhydrous Na<sub>2</sub>SO<sub>4</sub> and then centrifuged at 3000 rpm for 20 min. The fat layer was decanted, shaken well and centrifuged again before being filtered through Whatman filter paper containing sodium sulfate anhydrous to remove trace of water. The filtered samples were directly subject to analysis or kept in tightly closed container under a nitrogen blanket in -20°C.

### *Gas chromatography analysis*

Analysis of fatty acids composed of lard and palm oil were done using gas chromatography with flame ionization detector (GC-FID), according to AOCS official method Ce 1c-89 (1996) with slight modifications. Approximately 100 mg of samples (lard or palm oil) were dissolved in 1.0 ml hexane and added with 0.2 ml 1 M NaOCH<sub>3</sub> in methanol. The mixture was vigorously shaken for 1 min with a vortex mixer, added with 5 drops of saturated NaCl and mixed again using vortex for 15 sec. Subsequently, 1 µL of the clear supernatant was taken and injected into a gas chromatograph (Shimadzu GC-2010, Shimadzu Corp., Tokyo, Japan). The column used is RTX-5 capillary column (0.25 mm internal diameter, 30 m length, and 0.2 µm film thickness; Restex Corp., Bellefonte PA), oven was set at 50°C (hold for 1 min), then increased to 180°C (8 °C/min), 180 to 240°C (8°C/min), and finally held at 240°C for 5 min, carrier gas of N<sub>2</sub>, at 6.8 mL/min, FID was set 240°C,

and injector temperature used was 240°C; split ratio (1: 20). Standard FAMES of 37 compounds (C4 to C24) (Sigma Chemicals, St. Louis, MO, USA) were used to identify the retention times. Quantification analysis of FA was performed using normalization internal technique.

### *Quantitative analysis of lard in palm oil*

For calibration model, a set of standards consisting of lard in palm oil was made by blending of both at concentration ranges of 1 - 60% (v/v) of lard in palm oil. For validation/prediction, a series of independent samples, which were different from calibration samples, were constructed. Lard, palm oil as well as their blends in the neat form were analyzed using FTIR spectrophotometer. The spectral regions where the variations among them were observed were chosen for developing multivariate analysis.

### *FTIR spectra measurement*

FTIR spectra was scanned using a FTIR spectrometer ABB MB3000 FTIR spectrometer (Clairet Scientific, Northampton, UK) equipped with DTGS detector with a resolution of 4 cm<sup>-1</sup>, number of scanning of 32 co-adding in the 400–4000 cm<sup>-1</sup> region. Spectra were acquired using Horizon MB FTIR software version 3.0.13.1 (ABB, Canada). The samples were placed in contact with horizontal attenuated total reflectance (HATR) element (ZnSe crystal) at controlled ambient temperature (20°C). All spectra were rationed against a background of air spectrum. After every scan, a new reference air background spectrum was taken. These spectra were recorded as absorbance values at each data point in triplicate.

### *Statistical analysis*

PLS was accomplished using Horizon MB FTIR software version 3.0.13.1 (ABB, Canada). The leave-one-out cross-validation procedure was used to verify the calibration model. The values of root mean square error of calibration (RMSEC) and coefficient of determination (R<sup>2</sup>) were used as the validity criteria for the calibration. The predictive ability of PLS calibration model was further used to calculate the validation or prediction samples.

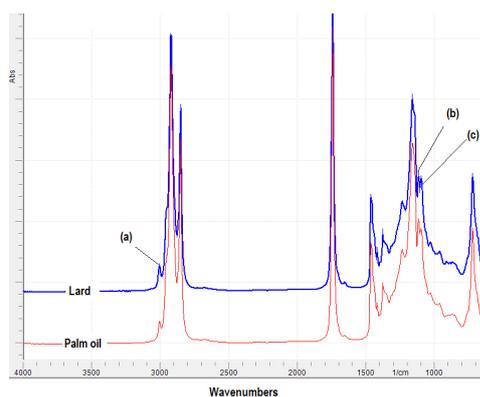
## Results and Discussion

### *FTIR spectra analysis*

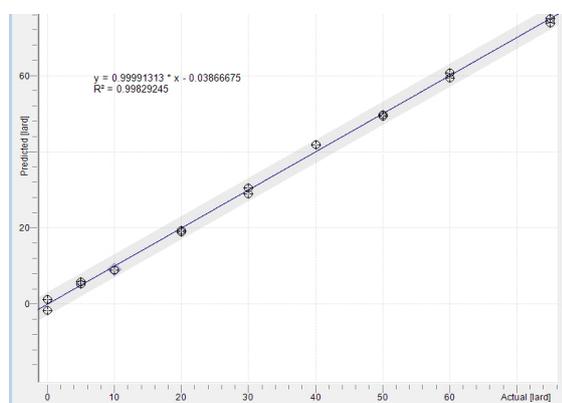
FTIR spectra can be used as a potential tool which allows one to make a first differentiation among fats and oils due to its capability as fingerprint technique. Figure 2 shows FTIR spectra of lard and palm oil exhibiting the characteristic peaks of edible fats

and oils spectra as described by Guillen and Cabo (1997) because the main components of fats and oils are triacylglycerols. Lard can be differentiated from palm oil by investigating the position of fatty acids, saturation level of the chains and the specific minor components present in the fats and oils. Table 1 listed the fatty acid composition of lard and palm oil.

Figure 1 showed FTIR spectra of lard and palm oil scanned at mid infrared region of 4000 – 650  $\text{cm}^{-1}$ . The analysis of functional groups responsible for IR absorption in fat and oil samples can be found elsewhere (Lerma-Garcia *et al.*, 2010; Vlachos *et al.*, 2006; Guillen and Cabo, 1997). Both spectra appear very similar, however, they revealed slight differences in terms of band intensities and the exact frequencies at which the maximum absorbance were generated in each fats and oils, due to the different nature and composition of evaluated fats and oils (Guillen and Cabo, 1997), especially at wavenumber regions of 3006 (a), 1117 (b) and 1098  $\text{cm}^{-1}$  (c).



**Figure 1.** FTIR spectra of lard and palm oil at mid infrared region (4,000 – 650  $\text{cm}^{-1}$ )



**Figure 2.** The relationship between actual value (*x*-axis) and FTIR predicted value of lard (*y*-axis) in palm oil at frequency region of 3006  $\text{cm}^{-1}$ . Frequency region of 3006  $\text{cm}^{-1}$  was attributed from cis-olefinic C-H, while frequencies of 1117 and

1098  $\text{cm}^{-1}$  come from the stretching vibration of ether linkage in triacylglycerols (Guillen and Cabo, 1997). Determination of fatty acid composition of studied samples (palm oil and lard) revealed that both oils have quite high amount of oleic acid (Table 1). However, compared to palm oil, lard contained linolenic acyl groups as twice as palm oil. This was reflected in the lard spectrum, in which sharper band was observed at frequency 3006  $\text{cm}^{-1}$  compared to palm oil spectrum. Fats with high proportion of linolenic and linoleic acyl groups show higher frequency for this band compared to high proportion of oleic acyl groups (Che Man *et al.*, 2012).

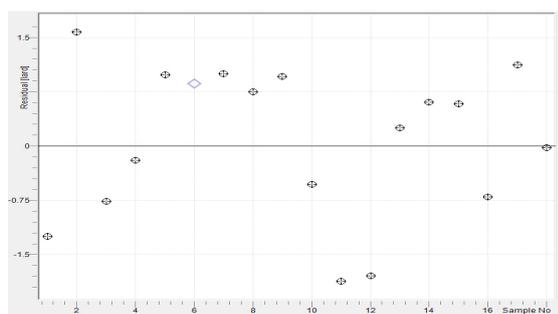
**Table 1.** Fatty acid composition of lard and palm oil

	Lard	Palm oil
C12:0		0.26 ± 0.02
C14:0	1.30 ± 0.02	1.01 ± 0.01
C16:0	20.66 ± 0.20	39.33 ± 0.12
C16:1	1.98 ± 0.01	0.12 ± 0.01
C17:0	0.48 ± 0.02	-
C18:0	10.91 ± 0.09	6.00 ± 0.76
c18:1 n9	39.12 ± 0.07	40.13 ± 0.66
C18:2n6	19.56 ± 0.30	10.46 ± 0.09
C18:3n6	1.22 ± 0.03	0.38 ± 0.00
C20:0	0.15 ± 0.01	0.22 ± 0.01
C20:1 n9	0.97 ± 0.03	0.15 ± 0.01
C22:0	0.03 ± 0.00	0.03 ± 0.02
C22:1	0.14 ± 0.01	0.01 ± 0.00

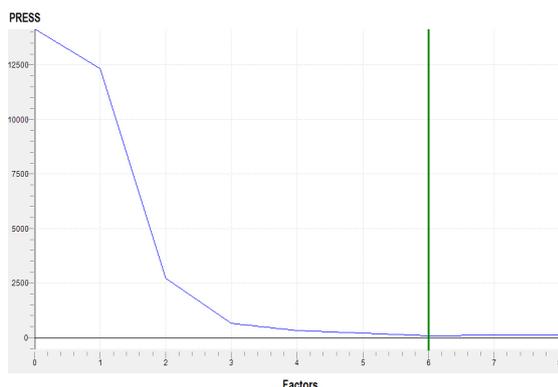
two peaks having maximum absorption at 1119 and 1100  $\text{cm}^{-1}$ , exactly at frequency regions of 1117 and 1098  $\text{cm}^{-1}$ . These peak heights were shown to be inversely related with proportion of saturated acyl groups and oleic acyl groups, respectively (Jaswir *et al.*, 2003). Lard had approximately equal proportion of saturated acyl groups and oleic acyl group which was reflected in the lard spectra, in which peaks of 1119 and 1100  $\text{cm}^{-1}$  appeared as having the same height. Palm oil on the contrary, having more concentration of saturated acyl groups than oleic acyl group, demonstrated unequal height of peak 1119 and 1100  $\text{cm}^{-1}$  in the palm oil spectrum. These wavenumbers in which the peak intensities (absorbancies) of lard and palm oil were slightly different were further selected to be optimized for analysis of lard in palm oil.

#### Quantitative analysis of lard in palm oil

Quantitative analysis of lard in palm oil was performed with the aid of partial least square (PLS) regression. In PLS calibration model, the absorbancies of lard with concentration level of 0.0 – 60.0 % (v/v) in palm oil were scanned at mid infrared region of



**Figure 3.** The residual analysis describing the difference between actual value and predicted value of lard in palm oil



**Figure 4.** The relationship between PRESS value and the number of factors

4,000 – 650  $\text{cm}^{-1}$ . PLS calibration was employed for making the relationship between actual value of lard ( $x$ -axis) and FTIR predicted value of lard ( $y$ -axis) in palm oil. Lard in palm oil was determined at wavenumbers of 1,480 – 1085  $\text{cm}^{-1}$ . The selection of frequency region is performed such a way that give the high value of  $R^2$  and the low value of RMSEC by taking into account the over-fitting, which may takes place during PLS calibration. Using PLS calibration model, the relationship between actual value and FTIR predicted value of lard in palm oil is shown in Figure 2. The residual analysis revealed that the errors occurring during analysis is random error, and there is no systematic error observed (Figure 3).

The performance of PLS calibration model was further assessed with cross validation using “leave-one-out” technique. Cross validation using “leave one out” technique was exploited to evaluate the performance of PLS model. The number of factors or principal components (PCs) used for developing PLS model was based on the predicted residual error sum square (PRESS) value (Sedman *et al.*, 1997). The PRESS value is a direct measure on how well a calibration predicts the concentrations left out during a cross validation (Smith, 2002). During cross validation the minimum value of PRESS value obtained was 99.04, achieved using 6 factors

or principle components (Figure 4). The root mean square error of cross validation (RMSECV) value obtained from cross validation of PLS calibration model was 2.87 % (v/v). This indicated that FTIR spectroscopy was reliable enough for the analysis of lard in palm oil.

## Conclusion

FTIR spectroscopy in combination with multivariate calibration of partial least square calibration has been successfully used for determination of lard in palm oil. The optimized frequency region used is 1480 – 1085  $\text{cm}^{-1}$ . The coefficient of determination obtained for the relationship between actual and FTIR predicted value was 0.998. The errors yielded during calibration and cross validation were relatively low, namely 1.69 % and 2.87 % (v/v), respectively. The developed method was rapid, no excessive sample preparation, and not involving the hazardous solvents and reagents.

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