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# The optimization of FTIR spectroscopy combined with partial least square for analysis of animal fats in quartenary mixtures

Abdul Rohman [a,b] and Yaakob B. Che Man [a,*]

[a] *Halal Products Research Institute, Universiti Putra Malaysia, Serdang, Selangor, Malaysia*
[b] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, and Halal Research Group LPPT, Gadjah Mada University, Yogyakarta, Indonesia*

**Abstract.** Four types of animal fats, namely lard (LD) and body fats of lamb (LBF), cow (Cow-BF) and chicken (Ch-BF), in quaternary mixtures were quantitatively analyzed using FTIR spectroscopy in combination with multivariate calibration of partial least square (PLS). The animal fats, either individual or in quaternary mixtures, were subjected to horizontal total attenuated total reflectance (HATR) as sample handling technique and scanned at mid-infrared region (4000–650 $cm^{-1}$) with resolution of 4 $cm^{-1}$ and with 32 interferograms. PLS calibration revealed that the first derivative FTIR spectrum was well suited for the correlation between actual value of LD and FTIR calculated value. The other animal fats (LBF, Cow-BF and Ch-BF) were better determined using normal FTIR spectra. The coefficient of determination ($R^2$) obtained using the optimized spectral treatments was higher than 0.99. The root mean standard error of calibration (RMSEC) values obtained were in the range of 0.773–1.55. Analysis of animal fats using FTIR spectroscopy allows rapid, no excessive sample preparation, and can be regarded as "green analytical technique" due to the absence of solvent and chemical reagent used during the analysis.

Keywords: Analysis, animal fats, FTIR spectroscopy, partial least square, quaternary systems

## 1. Introduction

Since the last decade, the exploitation of absorption spectroscopy including Fourier transform infrared (FTIR) spectroscopy, for quantitative analysis in the complex systems has experienced a considerable increase [12]. Analysis of fats and oils using FTIR spectroscopy can be considered as "green analytical chemistry" because this technique reduces or eliminates solvents and chemical reagents which are hazardous to human health or to environment [7,11].

Edible fats and oils are usually analyzed by determining the specific components such as triglyceride composition using reversed phase–high performance liquid chromatography and fatty acid profiles using gas–liquid chromatography rather than analysis of fats and oils as a whole matter [18]. Therefore, FTIR spectroscopy is developed in order to overcome this problem.

* Corresponding author: Y.B. Che Man, Halal Products Research Institute, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia. Tel.: +603 8943 0405; Fax: +603 8943 9745; E-mail: yaakobcm@gmail.com.

FTIR spectroscopy allows fast and non-destructive methods to quantitative analysis of fats and oils because this technique employs the simple mathematical treatments for the calibrations between concentrations of oils and the corresponding peak heights or areas as described in Lambert–Beer law [19]. However, in the oil mixtures in which the spectra of individual oils are very similar, it is necessity to use multivariate analysis to overcome this problem. Multivariate analysis can be used to extract subtle information from complex FTIR spectra that might contain overlapping peaks, interference bands and instrumental artifacts due to the measurement conditions [5]. From the several multivariate methods available, partial least square (PLS) has a large number of applications for quantitative analysis in the complex mixtures [8].

Currently, FTIR has been emerging technique for analysis of edible fats and oils due to its capability as "fingerprint technique", either for qualitative or quantitative purposes [14], especially in combination with multivariate calibration. PLS is one of the commonly used techniques in multivariate calibration and is based on the reduction of spectral data and inverse calibration. With PLS, it is possible to make a calibration for the desired component while completely modeling the other source of variations [13].

Our laboratory has developed FTIR spectroscopy in combination with multivariate calibrations of PLS to analyze palm kernel oil [10] and palm oil [15] in binary mixture with virgin coconut oil (VCO), extra virgin olive oil in palm oil [16], lard in cod liver oil [17] and lard in animal fats [3]. However, from literature search, there is no available report related to the application of FTIR spectroscopy for analysis of animal fats in quaternary systems. Therefore, this study highlighted the possibility of FTIR spectroscopy combined with multivariate calibration as rapid and reliable technique for quantitative analysis of animal fats in quaternary systems.

## 2. Material and methods

### *2.1. Sample preparation*

Adipose tissues of pig (lard), lamb, beef and chicken were obtained from various slaughtered house around Serdang, Selangor, Malaysia. Animal fats were obtained by rendering at 90–100°C for 2 h in the oven according to Rohman and Che Man [17]. The melted fats were strained through triple-folded Muslin cloth and dried by addition of anhydrous $Na_2SO_4$. Fats were subsequently subjected to centrifugation (3000 rpm, 20 min). The fat layer was decanted, shaken vigorously and then centrifuged again before being filtered using Whatman filter paper. The prepared oils were then used for FTIR and GC analyses.

### *2.2. Calibration*

For calibration, a training set of 30 samples consisting of lard (LD), body fats of beef (BFB), chicken (Ch-BF) and mutton (MBF) with certain concentration as shown in Table 1 is prepared. Each sample was subjected to FTIR analysis. Furthermore, a series of independent samples was also built for validation in order to evaluate the predictive ability of PLS model.

### *2.3. FTIR measurement*

A FTIR spectrometer of Nicolet 6700 from Thermo scientific (Madison, WI, USA) equipped with detector of deuterated triglycine sulfate (DTGS), beam splitter composed of KBr and OMNIC operating

Table 1
The composition of animal fats with certain concentrations used in PLS calibration

| Sample | Percentage of animal fats | | | |
|---|---|---|---|---|
| | LD | Cow-BF | Ch-BF | LBF |
| 1 | 0 | 100.0 | 0 | 0.0 |
| 2 | 100.0 | 0 | 0 | 0.0 |
| 3 | 0 | 0 | 100.0 | 0.0 |
| 4 | 0 | 0 | 0 | 100.0 |
| 5 | 97.5 | 2.5 | 0 | 0.0 |
| 6 | 95.0 | 1.25 | 3.75 | 0 |
| 7 | 90.0 | 2.0 | 3.0 | 5.0 |
| 8 | 87.5 | 3.75 | 7.5 | 1.25 |
| 9 | 85.0 | 5.0 | 6.25 | 3.75 |
| 10 | 80.0 | 7.5 | 10.0 | 2.5 |
| 11 | 1.25 | 60.0 | 23.75 | 15.0 |
| 12 | 2.5 | 75.0 | 12.5 | 10.0 |
| 13 | 5.0 | 85.0 | 2.5 | 7.5 |
| 14 | 7.5 | 75.0 | 5.0 | 12.5 |
| 15 | 10.0 | 52.5 | 17.5 | 20.0 |
| 16 | 15.0 | 10.0 | 50.0 | 25.0 |
| 17 | 20.0 | 15.0 | 15.0 | 50.0 |
| 18 | 3.75 | 2.5 | 1.25 | 92.5 |
| 19 | 25.0 | 0 | 20.0 | 55.0 |
| 20 | 30.0 | 0 | 70.0 | 0 |
| 21 | 55.0 | 3.75 | 40.0 | 1.25 |
| 22 | 60.0 | 1.0 | 36.5 | 2.5 |
| 23 | 50.0 | 0 | 50.0 | 0.0 |
| 24 | 45.0 | 1.0 | 1.5 | 52.5 |
| 25 | 40.0 | 3.75 | 55.0 | 1.25 |
| 26 | 22.5 | 7.5 | 17.5 | 52.5 |
| 27 | 0 | 50.0 | 50.0 | 0.0 |
| 28 | 42.5 | 2.5 | 3.125 | 1.875 |
| 29 | 35.0 | 7.5 | 32.5 | 25.0 |
| 30 | 2.5 | 92.5 | 1.25 | 3.75 |

system (version 7.0, Thermo Nicolet) was used for spectra measurements. Approximately of 1.0 ml oil was subjected to horizontal attenuated reflectance (HATR) accessory composed of zinc selenide (ZnSe) crystal. All spectral measurements were acquired over mid-infrared region (4000–650 $cm^{-1}$) with 32 interferograms co-added before Fourier transformation and at resolution of 4 $cm^{-1}$. Single-beam ATR spectra were collected and read as absorbance units using an air spectrum as a background.

### *2.4. Quantitative analysis*

Quantitative analysis of animal fats in quaternary systems was performed using PLS as described by Faber and Rajko [4]. The performance of calibration models were assessed by the coefficient of determination ($R^2$) and root mean standard error of calibration (RMSEC) values. Meanwhile, the validation model was assessed using root mean standard error of prediction (RMSEP) value which indicates how

well the developed model will perform the analysis of new samples; small RMSEP value shows that the concentration prediction of new sample has less error.

### *2.5. Fatty acid analysis*

Fatty acid composition of animal fats was determined as derivate of fatty acid methyl ester (FAME) according to Rohman and Che Man [15] using gas liquid chromatography with flame ionization detection (GC-FID). Quantification of each fatty acid was based on normalization area.

## 3. Results and discussion

### *3.1. Spectral analysis*

Fats and oils are typically consisted from fatty acid esters of glycerol and some minor components, therefore their FTIR spectra are almost similar [2]. The overlay of FTIR spectrum of evaluated oil samples (LD, LBF, Cow-BF and Ch-BF) at mid-infrared region (4000–650 $cm^{-1}$) is presented in Fig. 1. Each peak in FTIR spectra correlates with certain functional groups responsible for IR absorption and exhibits the characteristics bands for edible fats and oils.

The spectra of animal fats in Fig. 1 appear very similar, however, they reveal slight differences in term of band intensities and the exact frequencies at which the maximum absorbance are generated in each fats and oils, due to the different nature and composition of evaluated fats and oils [6], especially at 3007,

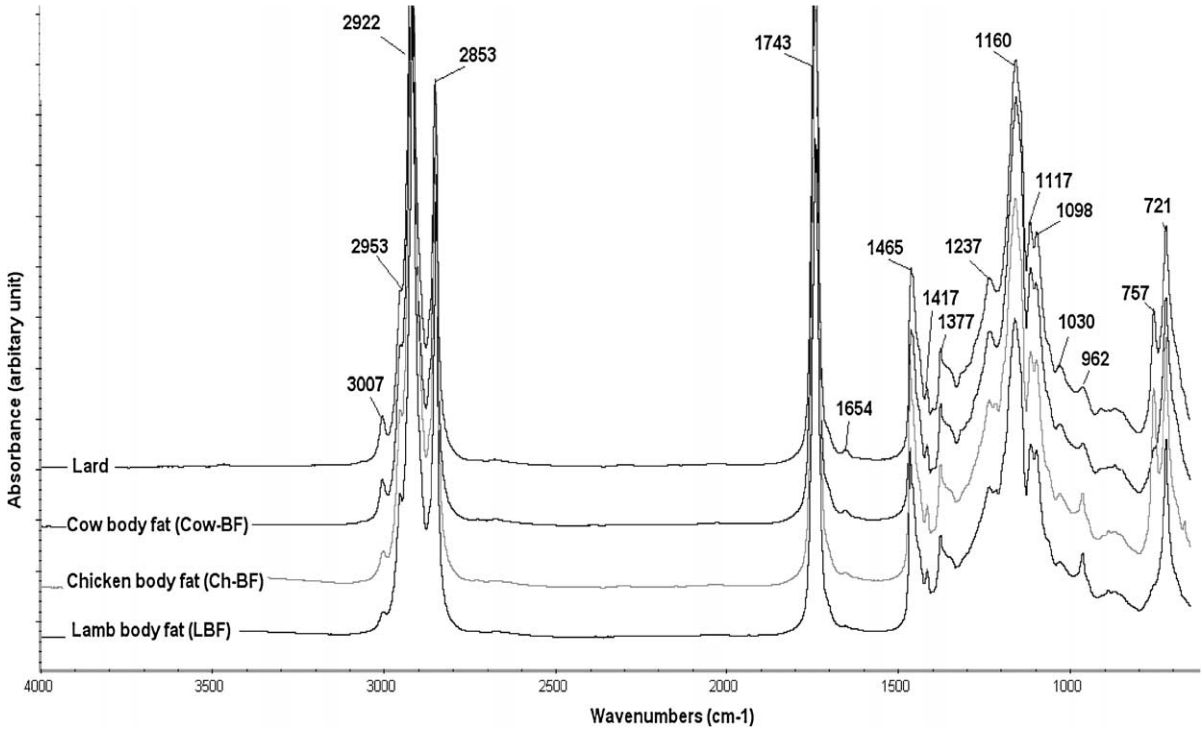


Fig. 1. FTIR spectra of studied animal fats which are scanned at mid-infrared region (4000–650 $cm^{-1}$).

Table 2
Fatty acid composition of lard, LBF, Ch-BF and Cow-BF determined using GC with flame ionization detection

| Fatty acid | Lard | LBF | Cow-BF | Ch-BF |
|---|---|---|---|---|
| (C10:0) | nd | 0.14 ± 0.00 | 0.06 ± 0.00 | nd |
| (C11:0) | nd | 0.14 ± 0.00 | 0.12 ± 0.00 | nd |
| (C12:0) | nd | 0.11 ± 0.00 | 0.08 ± 0.00 | 0.02 ± 0.00 |
| (C14:0) | 0.11 ± 0.03 | 2.64 ± 0.02 | 2.84 ± 0.05 | 0.86 ± 0.04 |
| (C14:1) | nd | 0.40 ± 0.01 | 0.65 ± 0.02 | 0.01 ± 0.00 |
| (C15:0) | 1.12 ± 0.02 | 0.70 ± 0.01 | 0.63 ± 0.01 | 0.02 ± 0.00 |
| (C15:1) | 0.08 ± 0.01 | 0.32 ± 0.00 | 0.03 ± 0.00 | 0.17 ± 0.02 |
| (C16:0) | 21.33 ± 0.89 | 21.49 ± 0.98 | 24.19 ± 0.01 | 28.27 ± 0.13 |
| (C16:1′) | 1.65 ± 0.24 | 1.23 ± 0.15 | 3.27 ± 0.04 | 6.10 ± 0.09 |
| (C17:0) | 0.46 ± 0.08 | 2.03 ± 0.03 | 1.31 ± 0.02 | 0.02 ± 0.00 |
| (C17:1) | nd | 0.62 ± 0.00 | 0.95 ± 0.01 | 0.12 ± 0.01 |
| (C18:0) | 11.39 ± 0.68 | 27.84 ± 0.42 | 16.47 ± 0.36 | 9.49 ± 0.23 |
| (C18:1n9) | 41.01 ± 2.28 | 30.06 ± 0.36 | 40.46 ± 0.31 | 38.33 ± 0.12 |
| (C18:2n6) | 17.65 ± 3.33 | 4.90 ± 0.14 | 4.44 ± 0.40 | 14.19 ± 0.13 |
| (C20:0) | 0.91 ± 0.03 | 0.55 ± 0.01 | 0.12 ± 0.00 | 0.15 ± 0.02 |
| (C18:3n6) | 0.97 ± 0.06 | 0.37 ± 0.02 | 0.12 ± 0.11 | 0.63 ± 0.03 |
| (C20:1) | 0.82 ± 0.17 | 1.47 ± 0.01 | 0.07 ± 0.02 | 0.10 ± 0.01 |
| (C21:0) | nd | 0.28 ± 0.01 | nd | 0.28 ± 0.02 |
| (C20:2) | 0.19 ± 0.05 | 0.21 ± 0.02 | 0.12 ± 0.11 | 0.07 ± 0.00 |
| (C22:0) | 0.69 ± 0.28 | 0.65 ± 0.02 | 0.07 ± 0.02 | 0.11 ± 0.01 |
| (C20:3n6) | 0.63 ± 0.43 | 0.83 ± 0.01 | 0.04 ± 0.01 | 0.08 ± 0.00 |
| (C22:1n9) | 0.10 ± 0.04 | 0.17 ± 0.00 | 0.04 ± 0.02 | nd |
| (C20:4n6) | 0.13 ± 0.04 | 0.04 ± 0.02 | nd | nd |
| (C22:2) | 0.10 ± 0.03 | 0.03 ± 0.01 | 0.02 ± 0.01 | nd |
| (C24:0) | 0.05 ± 0.02 | 0.08 ± 0.02 | 0.13 ± 0.00 | 0.02 ± 0.00 |

2922 and 2952 $cm^{-1}$ as well as at 1237, 1117 and 1098 $cm^{-1}$. For example, frequency at 3007 $cm^{-1}$ was attributed to –C=CH (*cis* double bond stretching) and can be correlated to mono-unsaturated fatty acids (MUFA). Fatty acid compositions of lard (LD) and body fats of lamb, cow and chicken (LBF, Cow-BF and Ch-BF) were compiled in Table 2. The MUFA contents of animal fats can be correlated with the band intensities at 3007 $cm^{-1}$. The high contents of MUFA in animal fats were followed with the increase of peak intensities at 3007 $cm^{-1}$. The order of peak intensities at 3007 $cm^{-1}$ is as follows: lard $>$ Cow-BF $>$ Ch-BF $>$ LBF, which is in agreement with the content increase of MUFA. Furthermore, these frequencies, in which the different peak absorption intensities of animal fats were observed, were selected to be optimized for analysis of animal fats using FTIR spectroscopy. The analysis of functional groups responsible for IR absorption in fat/oil samples can be found elsewhere [9,14].

## 4. Spectral region selection

Spectral region (frequency) selection is the major problem in FTIR analysis because the chosen frequency regions must be chosen in such a way that the ones describe the most characteristics analytes to be determined and to provide non-interfered data for the analytes [1]. After being selected, the spectral regions were further used for making a PLS calibration.

Table 3
$R^2$ values for PLS model using different spectral frequencies for analysis of oils in ternary mixtures

| Frequencies regions ($cm^{-1}$) | $R^2$ values for ternary mixtures of lard, LBF, Cow-BF and Ch-BF* | | | |
|---|---|---|---|---|
| | Lard | LBF | Cow-BF | Ch-BF |
| 4000–650 | 0.9834 | 0.7849 | 0.4881 | 0.6279 |
| 3050–2825 | 0.9893 | 0.7747 | 0.9901 | 0.4783 |
| 1500–1000 | *0.9995* | *0.9996* | *0.9996* | *0.9980* |
| 3050–2825 and 1500–1000 | 0.9984 | 0.7977 | 0.9980 | 0.5038 |

*Note*: *Spectral treatments selected for quantification are marked with italic.

Table 4
The statistical parameters using PLS calibration at frequency region of 1500–1000 $cm^{-1}$ for determination four animal fats in the quaternary mixture systems*

| Analytes of interest | Spectral treatments | Number of factor | Calibration performance | |
|---|---|---|---|---|
| | | | $R^2$ | SEC |
| Lard | Normal | 10 | 0.9995 | 1.07 |
| | First derivative | 10 | *0.9997* | 0.773 |
| | Second derivative | 10 | 0.9567 | 7.51 |
| LBF | Normal | 6 | *0.9996* | 0.912 |
| | First derivative | 10 | 0.9969 | 2.13 |
| | Second derivative | 10 | 0.9995 | 0.992 |
| Cow-BF | Normal | 10 | *0.9996* | 0.936 |
| | First derivative | 7 | 0.9856 | 5.49 |
| | Second derivative | 8 | 0.9946 | 3.36 |
| Ch-BF | Normal | 10 | *0.9980* | 1.55 |
| | First derivative | 5 | 0.9571 | 7.56 |
| | Second derivative | 1 | 0.4433 | 22.1 |

*Note*: *Spectral treatments selected for quantification are marked with italic.

PLS can be considered as full spectrum method, therefore it can be applied for analysis of component of interest for the whole FTIR spectral regions, rather than the specific regions [4]. For these reasons, several FTIR spectral regions and its combinations as shown in Table 3 were used for developing PLS calibration model. The selection of frequency regions was based on the highest values of coefficient of determination ($R^2$) and the lowest values of root mean standard error of calibration (RMSEC). Table 3 revealed the performance of PLS calibration for analysis of four animal fats in the quaternary mixtures in term of $R^2$ and RMSEC values.

From Table 3, it can be shown that frequency region at 1500–1000 $cm^{-1}$ is suitable for the analysis of four animal fats using PLS calibration. The use of single spectral region allows fast analysis because all animal fats evaluated can be determined at the same frequency region. The next step of optimization was further carried out by investigating the spectral treatments (normal or derivatives) using frequency 1500–1000 $cm^{-1}$. Again, the values of $R^2$ together with RMSEC were used for calibration criteria. These values, together with the number of factors used in such a calibration, are shown in Table 4.

Relying the highest values of $R^2$ and the lowest values of RMSEC as shown in Table 4, lard was determined using first derivative spectra at frequency region of 1500–1000 $cm^{-1}$, meanwhile normal

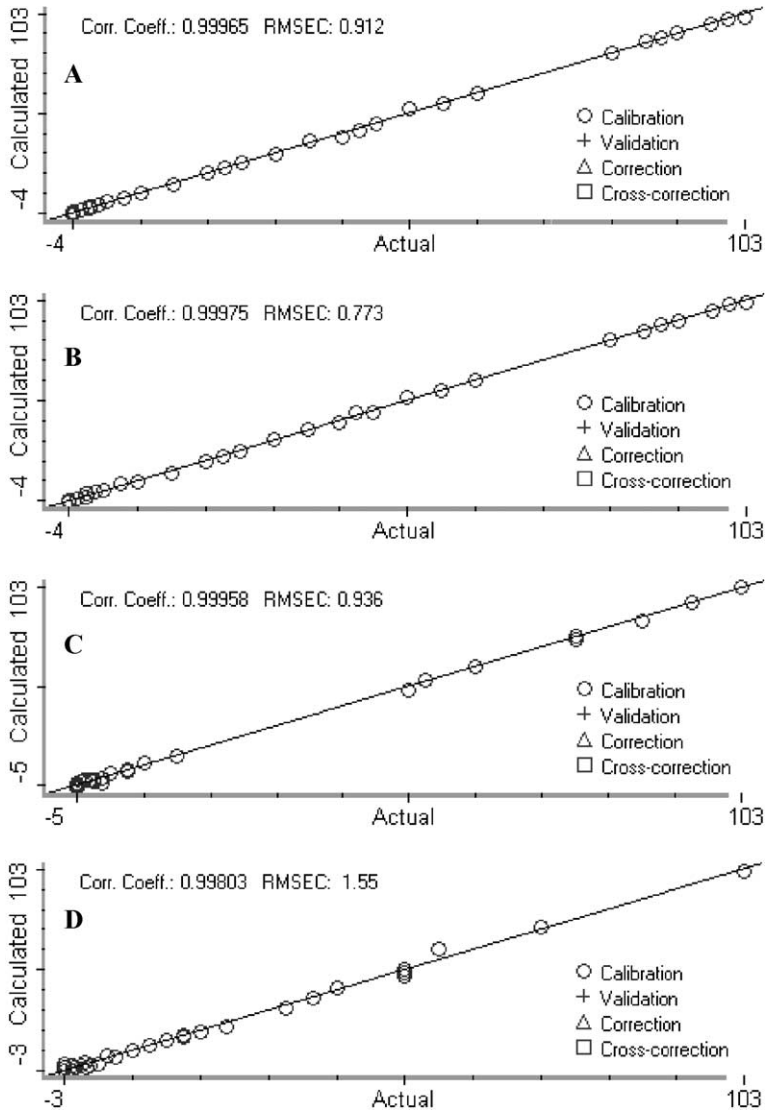


Fig. 2. Scatter plot for the relationship between actual value of animal fats and FTIR calculated value. A – lard; B – LBF; C – Cow-BF and D – Ch-BF.

spectra were selected for simultaneous determination of LBF, Cow-BF and Ch-BF. The scatter plot for relationship between actual lard value and FTIR predicted value of lard measured using first derivative FTIR spectra is exhibited in Fig. 2A; furthermore, the actual value of other animal fats (LBF, Cow-BF and Ch-BF) was also correlated altogether with FTIR predicted values using normal spectra as shown in Fig. 2B–D.

The calibration model was further validated using "leave-one-out" technique and the $R^2$ as well as RMSEP values were used as validity criteria. Table 5 compiled these values together with the equation obtained from the relationship between actual animal fats ($x$-axis) and FTIR predicted values ($y$-axis) at frequency regions of 1500–1000 $cm^{-1}$ using the optimized FTIR spectral treatments.

Table 5
The performance of validation model ($R^2$, RMSEP and equation obtained) used for prediction of animal fats

| Analytes of interest | Spectral treatments | Calibration performance | | |
|---|---|---|---|---|
| | | Equation | $R^2$ | RMSEP |
| Lard | First derivative | $y = 0.982x + 0.813$ | 0.989 | 3.97 |
| Cow-BF | Normal | $y = 1.014x - 1.594$ | 0.989 | 3.95 |
| Ch-BF | Normal | $y = 0.973x + 3.009$ | 0.968 | 5.14 |
| LBF | Normal | $y = 1.041x + 1.147$ | 0.962 | 4.39 |

## 5. Conclusions

We concluded that Fourier transform Infrared (FTIR) spectroscopy combined with ATR and PLS calibration can be used to analyze four animal fats at frequency region of 1500–1000 $cm^{-1}$. The developed method was rapid; with a total analysis time at about 3 min for each measurement, and it is taken into account as green analytical chemistry.

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