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FTIR spectroscopy combined with chemometrics for analysis of lard in the mixtures with body fats of lamb, cow, and chicken

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Abstract: Fourier transform infrared (FTIR) spectroscopy combined with chemometrics of partial least square (PLS) and discriminant analysis (DA) has been developed for simple analysis of lard in the mixtures with body fats of lamb (LBF), cow (Cow-BF), and chicken (Ch-BF). The spectral bands correlated with lard, LBF, Cow-BF and Ch-BF as well as their lard blends were scanned, interpreted, and identified. Qualitative differences among FTIR spectra are proposed as a basis for differentiating between the lard and its blends. DA with Mahalanobis distance principle in entire range of mid infrared (3300 – 650 cm^{-1}) was successfully provide an alternate method to differentiate lard and that in the mixtures with LBF, Cow-BF, and Ch-BF. Quantitative analysis using PLS calibration model is proposed to measure the percentages of lard in LBF, Cow-BF, and C-BF at selected fingerprint region (1500 – 900 cm^{-1}). The equation obtained between actual lard concentration in the mixture with LBF and FTIR predicted concentration in calibration model is $y = 0.995\ x + 0.098$ with coefficient of determination (R^2) was 0.995 and root mean standard error of calibration (RMSEC) of 0.98. The actual percentages of lard mixed with Cow-BF and Ch-BF were also correlated to FTIR predicted percentages at 1500 – 900 cm^{-1} using equations of $y = 0.999x + 0.016$ ($R^2 = 0.999$, RMSEC = 0.61); and $y = 1.002x + 0.034$ ($R^2 = 0.998$, RMSEC = 0.73), respectively.

Keywords: FTIR, lard, body fats, discriminant analysis, partial least square

Introduction

Lard is defined as "the fat rendered from fresh, clean, sound fatty tissues from swine (*Sus scrofa*) in good health, at the time of slaughter, and fit from human consumption" (Codex Allimentarius, 1991). It is appreciated as an important ingredient for cooking. Its production has been estimated more than 5.4 million tons annually, with China, USA, and Germany are the main producing countries (De Leonardis *et al*., 2007).

In some countries, food producers prefer to mix vegetable oils with lard to reduce the production cost. Currently, lard is one of the cheapest oils and is commonly available for the food industries. Lard or industrially modified lard could be effectively mixed with other vegetable oils to produce shortenings, margarines and other food oils (Marikkar *et al*., 2005). The presence of lard in any food systems is serious problems in view of religious concerns, because some religions like Islam, Judaism and Hinduism forbid their followers to consume any foods containing porcine and its derivatives (Regenstein *et al*., 2003).

Analysis of edible fats and oils are usually performed by determination of specific components present in fats or oils such as derivate of fatty acid methyl esters (FAME) using gas chromatography and triglyceride (TG) compositions with liquid chromatography (Rohman *et al*., 2010). Gas-liquid chromatography (GLC), combined with pancreatic lipolysis, and chemometrics of multivariate data analysis has been used for identification of lard in some vegetable oils by monitoring the changes of fatty acid composition in those vegetable oils, especially in *sn-2* position. Using GLC, 2% of lard in some vegetable oils can be detected (Luddy *et al*., 1964; Marikkar *et al*., 2005). Analysis of TG composition showed that both genuine and randomized lard had six dominant TG (C46, C48, C50, C52, C54, and C56) with quite different concentrations. TG with C52 represents the major constituent of genuine and randomized lard (Rashood *et al*., 1996). Using HPLC, the amounts of TG containing saturated fatty acid at C-2 position of lard were larger than those of other meat fats (Saeed *et al*., 1989). It is reported that using this technique, a level of 5 % lard can be detected in meat products

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(Rashood *et al*., 1996).

Lard has also been analyzed using differential scanning calorimetry (DSC). Tan and Che Man (2000) showed that the DSC heating and cooling curves of edible oils can be used in qualitative and quantitative ways for identification of edible oils. This method can detect 1 % w/w lard and randomized lard in RBD palm oil (Marikkar *et al.,* 2001). Most of methods described above are time consuming and not practical to be performed. Therefore, some efforts have been striven to develop a rapid and reliable technique for lard determination.

FTIR spectroscopy connected to multivariate calibration has been used for analysis of VCO in binary mixtures with palm kernel oil (Manaf *et al*., 2007), palm oil (Rohman and Che Man, 2009[a]), and olive oil (Rohman *et al*., 2010) and to analyze extra virgin olive oil mixed with palm oil (Rohman and Che Man, 2010). A level of 3-4 % of lard can be detected in food products (Che Man *et al*., 2005; Syahariza *et al*., 2005).

Che Man and Mirghani (2001) have developed FTIR spectroscopic method to analyze the presence of lard in body fats of chicken, lamb, and cow. Furthermore, Jaswir *et al*. (2003) has used FTIR in combination with PLS for analysis of lard in the mixture with body fat of mutton at frequency of 3010-3000, 1220-1095, and 968-965 cm^{-1} and at frequency 1419-1414 and 968-965 cm^{-1} for detecting lard in the mixture with body fat of cow. Both of the group researchers have used the different frequencies rather than using one frequency region for analysis of lard in certain blends with other body fats. Besides, they did not perform discriminant analysis (DA) in order to classify pure lard and that in the mixture with other body fats of lamb (LBF), chicken (Ch-BF), and cow (Cow-BF). Therefore, this study was conducted to investigate the possibility of detection of lard mixed with other animal fats such as LBF, Cow-BF, and Ch-BF using FTIR spectroscopic techniques at one frequency regions combined with chemometrics of DA and partial least square.

In fats and oils, DA has been used to differentiate among 10 different edible oils and fats, using FTIR, FT-near infrared and FT-Raman spectroscopy (Yang *et al*., 2005), virgin coconut oil and other vegetable oils (Manaf *et al*., 2007), olive oil (Fragaki *et al*., 2005), lard in admixtures with animal fats by HPLC (Marikkar *et al*., 2005), and to differentiate four vegetable oil types (cottonseed, peanut, soybean and canola) by near infrared reflectance spectroscopy (Bewig *et al*., 1994).

Materials and Methods

Materials

Adipose tissues of pig (lard), lamb, beef and chicken were obtained from various slaughter houses around Serdang, Selangor, Malaysia. Animal fats were prepared according to Rohman and Che Man (2009[b]).

Discriminant analysis

Lard was mixed with body fats of lamb (LBF), cow (Cow-BF) and chicken (Ch-BF) in order to obtain a series of standard or trained sets of 10 pure and 20 samples containing 1–50% v/v of lard in LBF, Cow-BF, Ch-BF. The samples containing body fats were assigned as adulterated, while a series of pure lard was marked lard and classified using FTIR spectra.

Quantitative analysis of lard

Quantitative analysis of lard in body fats were performed using partial least square (PLS) calibration. PLS calibration was carried out by preparing a set of 20 standards consisting of lard in LBF, Cow-BF and Ch-BF ranged from 1.0 to 50.0 % v/v. For validation, 32 independent samples were constructed. All samples were analyzed using FTIR spectrometer. The spectral regions at whole frequency regions and at selected fingerprint regions were chosen for optimization the calibration and validation models.

FTIR instrumental analysis

FTIR spectrometer Nicolet 6700 (Thermo Nicolet Corp., Madison, WI) equipped with a detector of deuterated triglycine sulphate (DTGS) and connected to software of OMNIC operating system (Version 7.0 Thermo Nicolet) was used to obtain FTIR spectra of samples. The samples were placed in contact with attenuated total reflectance (ATR) element (ZnSe crystal) at controlled ambient temperature. FTIR spectra were collected in frequency 4000 – 650 cm^{-1} by co-adding 32 scans and at resolution of 4 cm^{-1}. 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

The software TQ Analyst^{TM} (Thermo electron Corporation) was used for DA and PLS. For PLS, the leave-one-out cross-validation procedure was used to verify the calibration model. The values of root mean standard error of calibration (RMSEC) and coefficient of determination (R^2) were used as the validity criteria for the calibration. The validation was further investigated using the mean difference (MD) and standard deviation of difference (SDD) for accuracy and reproducibility.

Results and Discussion

The TGs are the major component of fats and oils, therefore they dominate spectra. Figure 1 shows the FTIR spectra of lard, LBF, Cow-BF, and Ch-BF. These spectra are very similarly and showed a typical characteristic of absorption bands for common TG (Safar *et al*., 1994). FTIR spectroscopy can be regarded as a potential analytical technique offering a differentiation between lard and LBF, Cow-BF, and Ch-BF because of its ability as "fingerprint" technique. Peak in the region 3007 cm^{-1} is due to C-H stretching vinylic. The strectching vibrations of methylene ($-CH_2-$) and methyl ($-CH_3$) groups are observable in frequencies 2922 and 2852 cm^{-1}, respectively. Methylene and methyl groups are also observable at regions of 1465 cm^{-1} and 1375 cm^{-1} due to their bending vibrations (Pavia *et al*., 2001). The carbonyl (C=O) absorption of ester linkage is observed at frequency 1740 cm^{-1} with strong intensity due to the great difference of electro-negativity of carbon and hydrogen atoms. The bands at 1235, 1160, 1117, 1098 and at 721 cm^{-1} are results from the overlapping of the methylene rocking vibrations and the out of plane bending vibration of *cis*-disubstituted olefins (Guillen and Cabo, 1997). From the point of view of spectra observations with naked eye, it is very difficult to differentiate lard and LBF, Cow-BF, and Ch-BF. However, a precise investigation in fingerprint region, especially in wavenumbers of 1500 – 1000 cm^{-1}, revealed that there are visual differences at absorption peaks at 1162 cm^{-1} (**a**) and two adjacent peaks at 1117 (**b**) and 1097 cm^{-1} (**c**). An absorbance ratio at 1117 and 1097 cm^{-1} (A_{1117}/A_{1097}) was used by Alam and Hamid (2007) to differentiate among vegetable oils. The value of A_{1117}/A_{1097} from lard and LBF, Cow-BF, and Ch-BF is shown in Table 1. These values for pure animal fats are almost constant. If fats are mixed each other, these values will be changed according to type and amount of added fats. In this study, the chemometric techniques namely discriminant analysis (DA) was used for lard differentiation. Furthermore, the level of lard mixed with each other can be quantitatively determined using partial least square (PLS) technique.

Table 1. Ratio value of A_{1117}/A_{1097} of lard and body fats of lamb (LBF), cow (Cow-BF), and Chicken (Ch-BF)

Animal fats	A_{1117}/A_{1097}
Lard	0.977
LBF	0.937
Cow-BF	0.925
Ch-BF	0.931

Discriminant analysis

As one of the classification analysis, DA is designed to find the mathematical models capable of recognizing the membership of each object to its proper class on the basis of a set of FTIR spectra. Once a classification model has been obtained, the membership of unknown objects to one of the defined classes can be predicted (Rohman and Che Man, 2009[a]).

Lard and lard in mixture with LBF, Cow-BF, and Ch-BF were classified into two groups, known as pure lard and lard in mixture of body fats. DA was applied to both classes in the wavenumber of 3,300–800 cm^{-1}. Figure 2 showed the Coomans plot for the classification of lard in mixture with LBF (A), Cow-BF (B), and Ch-BF (c). The *x*-axis shows Mahalanobis distance to lard, while the *y*-axis shows the distance to lard in mixture with body fats. The Mahalanobis distance is useful in assigning whether a set of unknown value samples is similar to a collection set of known measured samples. The Coomans plot clearly exhibits the separated group of lard and lard in mixture. In this study, the DA model revealed one misclassified from all samples because of the close similarities in the chemical composition between lard and one of samples misclassified.

Partial least square (PLS) model

PLS is relied on its ability to exploit FTIR spectral data from broad spectral frequencies and to correlate spectral changes in the concentration of a component of interest while simultaneously accounting for other spectral contributions that may perturb FTIR spectra (Syahariza *et al*., 2005). PLS calibration model was developed based on the calibration standard that included the different weighted amounts of lard blended with LBF, Cow-BF, and Ch-BF.

FTIR spectroscopy is regarded as "fingerprint" technique, especially in region of 1500 - 650 cm^{-1}. PLS is also called full spectrum method, therefore it can be applied for analysis of component of interest at the whole FTIR spectral regions rather than specific regions (Faber and Rajko, 2007). For these reasons, two FTIR spectral regions, namely using whole spectra (3300-700 cm^{-1}) and at selected frequency regions (1,500 – 900 cm^{-1}) were used for developing a 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

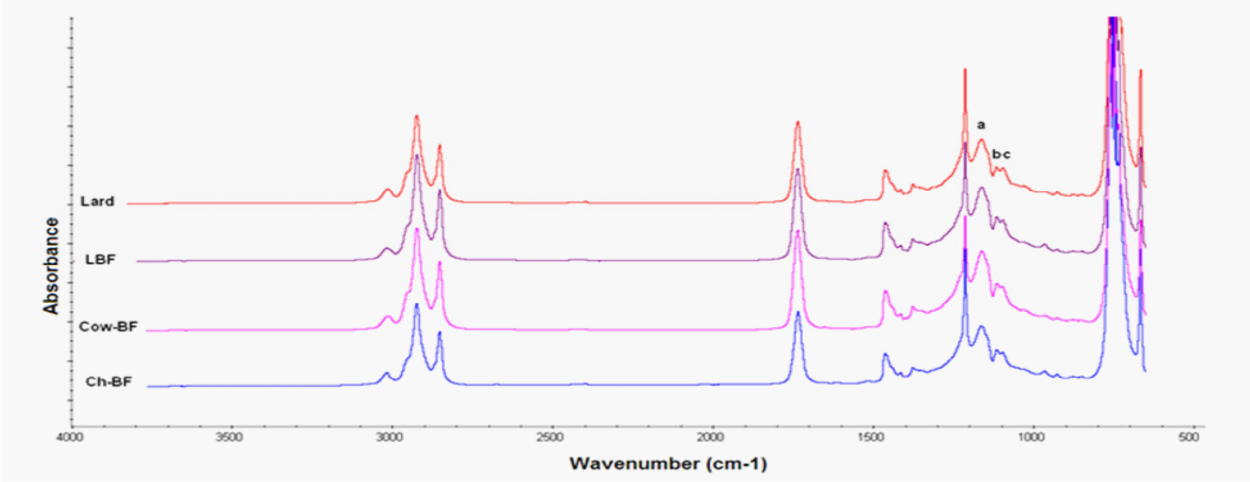

Figure 1. FTIR spectra of lard, LBF, Cow-BF, and Ch-BF at wavenumber 4000 – 650 cm^{-1}

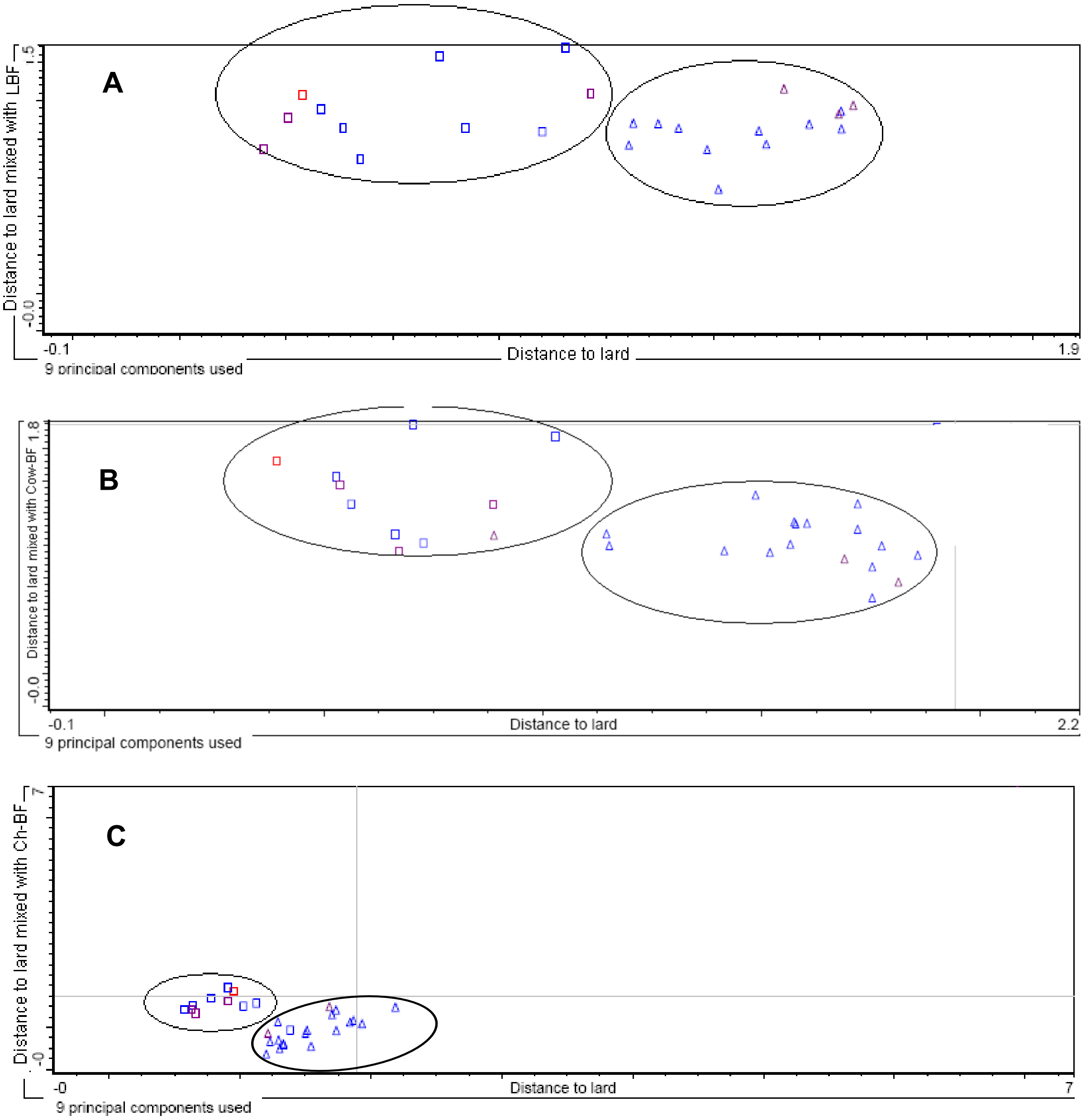

Figure 2. The cooman plot of lard and lard in mixture with LBF (A), Cow-BF (B), and Ch-BF (C): (□) lard; (Δ) lard in mixture.

mean standard error of calibration (RMSEC). Table 2 revealed the performance of PLS calibration for analysis of lard in the mixtures with LBF, Cow-BF, and Ch-BF in term of R^2 and RMSEC values.

Based on the highest values of R^2 and the lowest values of RMSEC as shown in Table 2, frequency region of 1500- 900 cm^{-1} was selected for quantification of lard in the mixtures with LBF, Cow-BF, and Ch-BF. The relationship between actual lard concentration against the PLS FTIR predicted in mixture with LBF, Cow-BF, and Ch-BF is shown in Figure 3 A, B, C, respectively. All equations revealed a good relationship between actual lard value and FTIR predicted values with R^2 greater than 0.99.

The calibration model was further cross validated by removing one standard at a time. The R^2 value of 0.993 ($y = 0.982x + 0.282$) for lard in mixture with LBF, R^2 of 0.991 ($y = 0.994x + 0.538$) for lard in mixture with Cow-BF and R^2 of 0.983 ($y = 0.933x + 1.855$) for lard in mixture with Ch-BF were obtained, respectively (Figure 4). Based on the calibration and vaidation curves, it can be stated that lard with level 1 % v/v was possible to be detected using FTIR spectroscopy in combination with multivariate calibration of PLS.

Table 3 shows the statistical results calculated from cross validation as mean difference (MD) and standard deviation of difference (SDD) for accuracy and reproducibility methods in the determination of lard in the mixtures with LBF, Cow-BF, and Ch-BF. Accuracy is a measure of the closeness of agreement between actual data and the predicted FTIR results. The low values of MD*a* (0.22, 0.24, and 0.32), and SDD*a* (1.56, 1.74, 1.97) for lard in the mixture with LBF, Cow-BF, and Ch-BF respectively, show that the FTIR is well suited for determining lard in the mixtures with body fats. Meanwhile, low MD*r* values (0.28, 0.27, 0.42) and SDD*r* (1.87, 1.92, 2.08), respectively, indicate that the FTIR method has appreciably high repeatability.

Conclusions

Fourier Transform Infrared (FTIR) Spectroscopy combined using attenuated total reflectance (ATR) sampling handling technique and chemometrics of DA and PLS can be used to analyze the lard contents in the mixtures with LBF, Cow-BF, and Ch-BF. The developed method was rapid, with a total analysis time less than 3 min for one measurement. Furthermore, it is environmentally friendly and the use of excessive time, chemical reagents and solvents can be avoided.

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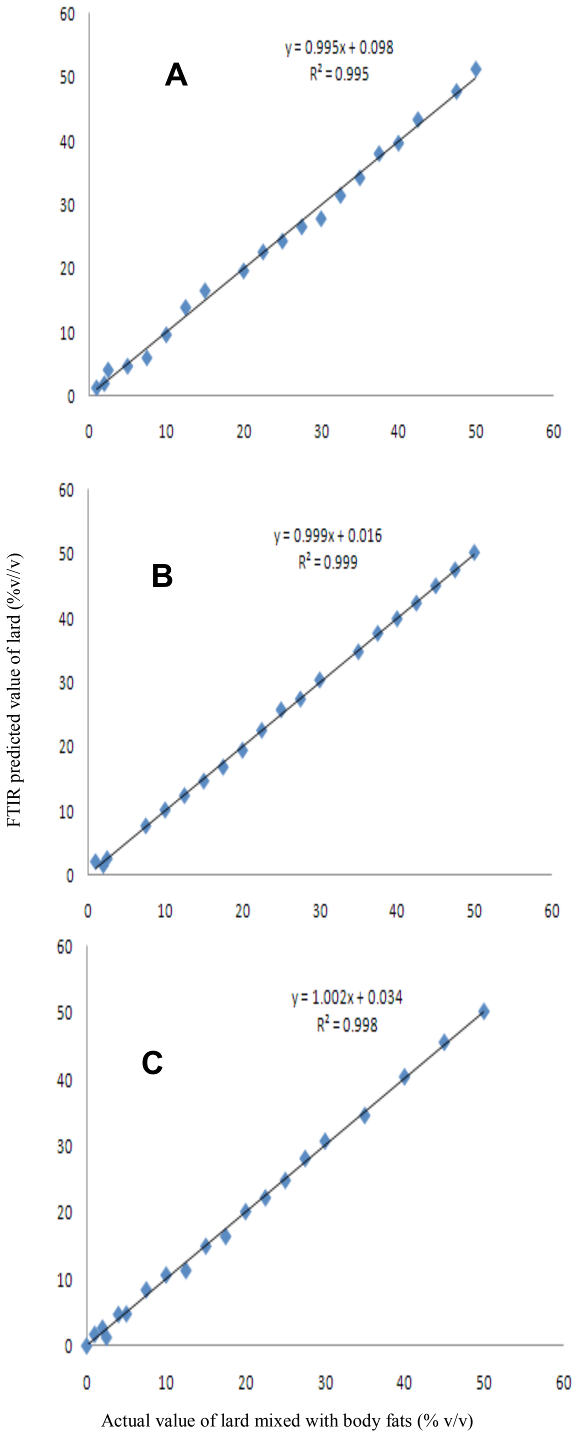

Figure 3. PLS calibration model for relationship between actual value of lard and FTIR predicted value in mixture with LBF (A), Cow-BF (B), and Ch-BF (C)

Table 2. R^2 and RMSEC values for analysis of lard in the mixtures with LBF, Cow-BF, and Ch-BF*

Analyte	Frequency regions (cm^{-1})	PLS performance	
		R^2	RMSEC
Lard in mixture with LBF	3100-650	0.897	4.37
	1500-800	0.995	0.98
Lard in mixture with Cow-BF	3100-650	0.997	1.23
	1500-800	0.999	0.61
Lard in mixture with Ch-BF	3100-650	0.796	9.05
	1500-800	0.998	0.73

Table 3. Accuracy and repeatability of FTIR spectroscopic technique for determination of lard contents in the mixtures with LBF, Cow-BF, and Ch-BF obtained by cross validation*

Statistics	LBF		Cow-BF		Ch-BF	
	Actual	FTIR	Actual	FTIR	Actual	FTIR
MD*a*	0.22		0.24		0.32	
SDD*a*	1.56		1.74		1.97	
MD*r*		0.28		0.27		0.42
SDD*r*		1.87		1.92		2.08

*MD, mean difference; SDD, standard deviation of difference; a, accuracy; r, repeatability.

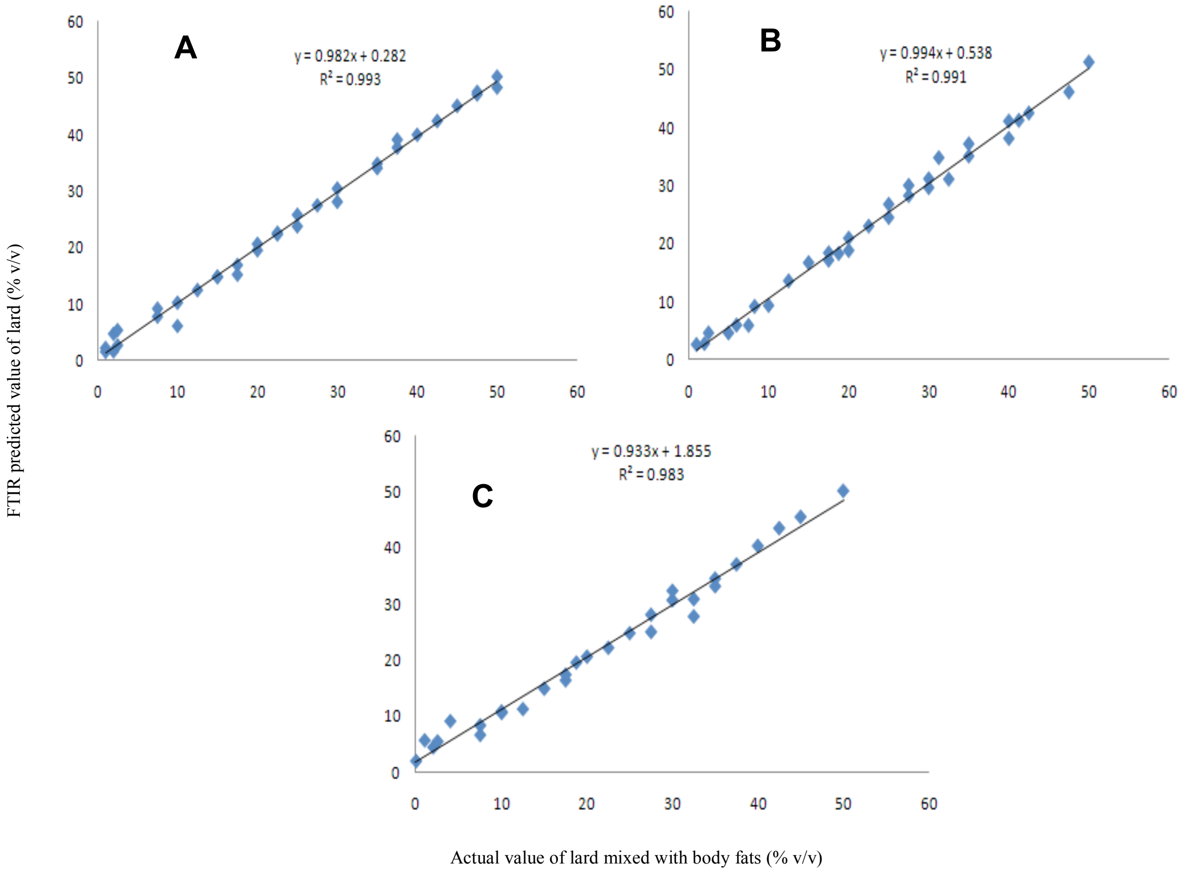

Figure 4. Relationship between actual value of lard and FTIR predicted value in mixture with LBF (A), Cow-BF (B), and Ch-BF (C) in validation

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