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Detection of lard adulteration in cake formulation by Fourier transform infrared (FTIR) spectroscopy

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Abstract

A rapid method was developed to determine the lard content in cake formulation using Fourier transform infrared (FTIR) spectroscopy. The lard was added to the shortening in cake recipe at 0–100% lard. FTIR spectra were recorded using attenuated total reflectance cell. A chemometric partial least squares was used to derive FTIR spectroscopic calibration model in regions of 1117–1097 and 990–950 cm^{-1}. The coefficient of determination (R^2) for the models was computed by comparing the results from the FTIR spectroscopy against the actual value. The R^2 obtained was 0.9790 with standard error (SE) of calibration was 1.7520. A validation approach was used to optimize the calibration and the R^2 of validation and SE of prediction were computed. Our results support the use of FTIR spectroscopy as an efficient and accurate method for detection lard in cake formulation.

Keywords: ATR; Cake formulation; FTIR spectroscopy; Lard and shortening

1. Introduction

Authenticity is an important food quality criterion. Regulatory bodies, food processor and consumers increasingly demand rapid methods for confirming food authenticity. Adulteration of food has ranged from the simple addition of natural compounds to the much more serious case of contamination with harmful substances (Defernez & Wilson, 1995). Numerous analytical methods have been used for the analysis of food adulterants such as differential scanning calorimetry (DSC), Fourier transform infrared (FTIR) spectroscopy, gas chromatography (GC), high pressure liquid chromatography (HPLC), nuclear magnetic resonance (NMR), and DNA-based method (Che Man & Mirghani, 2001; Coni, Pasquale, Cappolelli, & Bocca, 1994; Lipp, 1996; Lockley & Bardley, 2000; Rashood, Abou-Shaaban, Abdel-Moety, & Rauf, 1996; Wesley, Barnes, & Mc Gill, 1995). Ideally, the identification technique should be rapid, easy to use and of low cost.

Oils and fats have been liable to adulteration, to a greater or lesser degree since very early time. Adulteration is increasingly more difficult to detect when the contaminant has a composition approaching that of the original oil (Rossell, King, & Downes, 1983). Some manufacturers choose to blend lard or tallow with other vegetable oils to produce shortening, margarine and other specialty food oils (Gillies, 1974).

Food products containing pork and lard are of great concern to followers of Islamic and Orthodox Jewish religions. Both religions prohibit the consumption of pork and lard in any products (Al-Qaradawi, 1995; Regenstein, Chaudry, & Regenstein, 2003). Besides, diets rich in lard are known to associate with certain health risk such as hypercholesterolemia and coronary heart disease (Rashood et al., 1996). In view of

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the biological complications associated with pork and lard, and the restrictions on their consumption by some religions, a reliable method is required for the detection of lard in its various forms to monitor the production and authenticity of such products. Past studies on lard detection were primarily based on GC, HPLC, and DSC and their focus were mainly on raw or fresh product and some processed product (Coni et al., 1994; Marikkar, Ghazali, Long, & Lai, 2003; Rashood et al., 1996; Saeed, Abu Dagga, & Rahman, 1986).

Shortening are very important ingredient for the baking industry, due to the fact that they comprise from 10% to 50% of most baked products. It is a unique food ingredient, in that a high degree of interchangeability among the raw materials is possible for many products and uses. In most applications, shortening are multifunctional. Shortening provides a heat transfer medium, lubricity and flavour to fried foods; aeration, lubricity, and structure to cakes, icings, fillings, crème fillers, and whipped toppings, baked pastries, bread and sweet rolls. Therefore, shortening is a major contributor to the characteristic structure of most prepared food products and has a significant effect upon the finished product quality (O'Brien, 1998).

Lard and oleo (the liquid fraction of beef fat) are widely used in producing compound shortening, which is a mixture of animal fat and vegetable oil (O'Brien, 1998). Lard and lard compounds have been largely displaced in modern times by compound fats made from refined coconut oil, palm kernel oil, cotton seed oil, and other vegetable oils. The term compound fat was first given to mixtures of vegetable oils and animal fat, but today the modern product is almost universally referred to as shortening and sold under proprietary names. Commercial shortening are usually prepared by inter-esterification of the fat samples (Rashood et al., 1996).

Mid infrared spectroscopy has recently been applied to this problem using some experimental and statistical approaches. FTIR spectroscopy represents an important tool used for quality control and monitoring process in the food industry because it is less expensive, better in performance and easier to use than other method (Van de Voort, Sedman, & Ismail, 1993). FTIR spectroscopy offers a fast and non-destructive alternative to chemical measurement techniques for qualitative characterization and quantitative measurements. Infrared spectroscopy has been used to provide information on the molecular composition and structure of a diverse range of materials (Cronin & McKenzie, 1990; Guillen & Cabo, 1997a). In comparison with conventional instruments, FTIR has a higher energy throughput, excellent reproducibility and accuracy from the laser source. With the increasing use of computers, FTIR can easily manipulate spectral information, and its advance chemometric software is equipped to handle the calibration.

FTIR spectroscopy has shown to be useful for a range of adulteration problems in food sector such as in raspberry purees (Kemsley, Holland, Defernez, & Wilson, 1996), jam (Defernez & Wilson, 1995), extra virgin olive oil (Lai, Kemsley, & Wilson, 1995) and coffee (Briandet, Kemsley, & Wilson, 1996). FTIR also has been used in detecting the presence of lard in mixture of animal fats (Che Man & Mirghani, 2001), other vegetable fats in cocoa butter (Goodacre & Anklam, 2001), characterization of edible oils and lard (Guillen & Cabo, 1997b), and fatty acid composition of Spanish shortening (Alonso, Fraga, Juarez, & Carmona, 2002). Al-Jowder, Kemsley, & Wilson (1997) used the mid-infrared spectroscopy for addressing certain authenticity problems with selected fresh meats and reported about semi-quantitative analysis of meat mixtures.

The main objective of this study was to develop procedures for rapid detection of lard in food products, particularly in cake formulation and to determine the amount of the adulterant.

2. Materials and methods

2.1. Lard samples

Pure lard sample that was used as the shortening was extracted by rendering the adipose tissues from various part of slaughtered pigs, which was obtain from Seri Kembangan, Selangor, Malaysia. The fat was then filtered to a container, flushed with nitrogen gas and stored for further analysis.

2.2. Cake sample preparation

All ingredients (commercial shortening: mixture of partially hydrogenated soybean and cotton seed oils, flour, sugar, eggs and vanilla essence) purchased from local supermarket were used in the cake recipe. Lard was added to replace commercial shortening, ranging from 0 to 60 g (total fat: 60 g) with an interval 2 g. The cakes were baked at 250 °C for 40 min using electric oven (Pensonic, Penang, Malaysia). Total baked cakes were 31 samples.

2.3. Fat extraction

Bligh and Dyer extraction method using chloroform/methanol/water (2:1:1) vol/vol as a solvent with slight modification (Kinsella, Shimp, & Weihrauch, 1977; Manikariza, Covaci, & Schepens, 2001; Smedes & Thomasen, 1996) was used to extract the fat from all cake samples. The fat was stored under nitrogen blanket in the refrigerator for further FTIR analysis. All chemicals and solvents were of analytical grade.

2.4. Calibration samples

Eighteen from 25 fat samples extracted from the cakes have been used in this study as the matrices for developing the calibration and seven independent samples for validation, using chemometric partial least square (PLS). However, samples for validation set were selected, so that it will covers the whole range of concentration.

2.5. Instrumental analysis

The instrument used for this work was a Perkin–Elmer spectrum RXI FTIR spectrometer (Perkin–Elmer, Beaconsfield, Buckinghamshire) equipped with a LITA (Lithium tantalate) detector and controlled by a Pentium III PC run under Windows-based PE Spectrum Lite v 2.1. The software used for FTIR data collection was the Infrared Data Management System. The melted samples were placed in contact with the attenuated total reflectance (ATR) element (ZnSe crystal, 45° ends). FTIR data were collected from 32 scans at a resolution of 4 cm^{-1} and a gain of 1.0 and strong apodization throughout the region 4000–400 cm^{-1}. These spectra were ratioed against background air spectrum. After every scan, a new reference air background spectrum was taken. The ATR crystal was carefully cleaned between samples with hot water and acetone. The cleaned crystal was examined for spectral authenticity to ensure that no residue remained from the previous sample. These spectra were recorded as absorbance values at each data point, which were stored on the diskette for subsequent data analysis.

2.6. Statistical analysis

The relationships between each FTIR spectrum parameter and actual data were determined using the software spectrum Quant+ version 4.1 (Perkin–Elmer). Using the software, a PLS approach was choosen to develop a calibration model. The model was validated by the validation procedure. The power of PLS is based on its ability to use spectral information from broad spectral regions and to correlate spectral changes with changes in the concentration of a component of interest while simultaneously accounting for other spectral contributions that may perturb the spectrum model (Che Man, Setiowaty, & Van de Voort, 1999). A Microsoft Excel 98 spreadsheet was used to correlate the FTIR – predicted and actual data. The good correlation obtained for the calibration samples, indicated the adequacy of the FTIR calibration. Accuracy was assessed based on the smallest standard error of calibration (SEC) and the highest coefficient of determination (R^2) (Dubois, Van de Voort, Sedman, Ismail, & Ramasamy, 1996).

3. Results and discussion

The FTIR spectra afford information on the functional groups of the sample. In a previous paper, the assignment of the FTIR spectrum bands of edible oil and lard has been commented (Guillen & Cabo, 1997b). The spectra of fat from cakes with 0% lard, A, and fat from cakes with 100% lard, G, are shown in Fig. 1. The entire range of spectra looks almost similar for both the oils, unless one observes very closely. This is due to the similar chemical composition of the both oils.

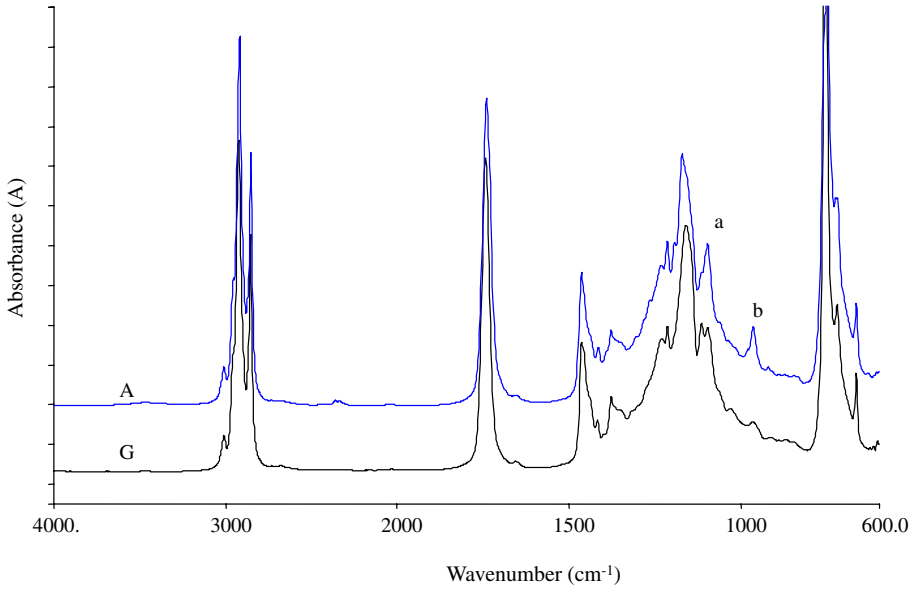

Fig. 1. FTIR spectra of fat extracted from cakes with (A) 0% lard and (G) 100% lard. The labeled peaks are absorption bands that are significant in differentiating between both spectra.

Fig. 1 illustrates the dominant spectral features associated with both of the spectra. The frequency range of 3009–2800 cm^{-1} is due to CH stretching absorptions, the carbonyl absorption of the triacylglycerol ester linkage at 1744–1739 cm^{-1}, the bands associated with the fingerprint region (1500–1000 cm^{-1}), C=C–H bending vibration of *trans* double bonds at 990–950 cm^{-1} and the overlapping of the methylene rocking vibration and the out-of-plane bending vibration of *cis*-disubstituted olefins at 723 cm^{-1} (Guillen & Cabo, 1997b).

Differences between the spectra of fat extracted from cakes with different proportion of lard/shortening are observed in region of 1117–1097 and 990–950 cm^{-1}. The two regions are illustrated in Fig. 2 as 'a' and 'b'.

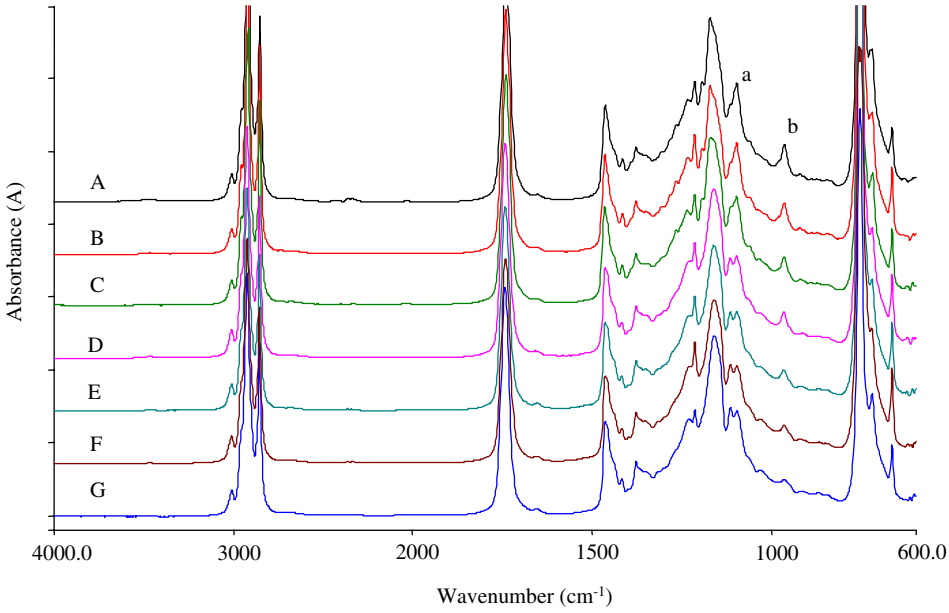

Fig. 2. FTIR spectra of fat extracted from cakes with (A) 0% lard, (B)–(F) lard/shortening mixture, and (G) 100% lard in region 4000–600 cm^{-1}. The percentage of lard in shortening mixtures are (B) 10 g = 17%, (C) 22 g = 33%, (D) 30 g = 50%, (E) 40 g = 66%, and (F) 50 g = 83% lard in shortening.

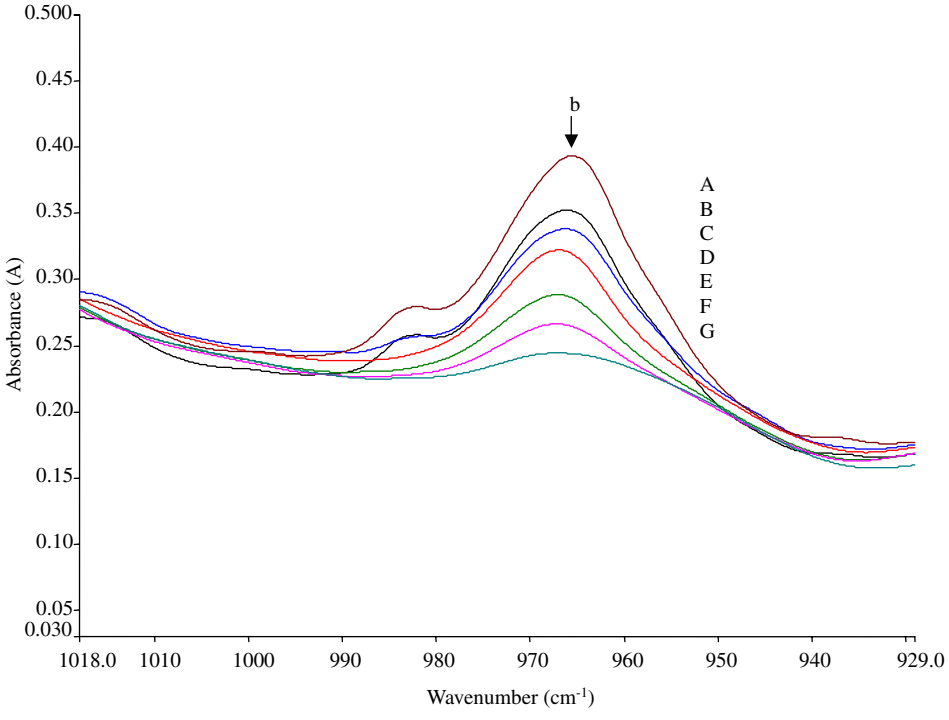

Fig. 3. FTIR spectra of fat extracted from cakes with (A) 0% lard, (B)–(F) lard/shortening mixture, and (G) 100% lard, illustrating changes in absorbance value of the band 'b' in region 990–950 cm^{-1}. See Fig. 2 for abbreviations and composition of the blends.

3.1. Frequency range 1117–1097 cm^{-1}

In this frequency range, the spectra of 100% lard, G showed two overlapping peaks having maxima at 1116.29 and 1097.49 cm^{-1}, where as spectra of 100% shortening (A) only one clear peak at 1097.48 cm^{-1}. These peaks have been found to be inversely related to the proportion of saturated acyl groups and oleic acyl groups, respectively, (Firestone & Sheppard, 1992). From the above results, it can be concluded that both lard and shortening contain saturated and oleic acyl groups in their structures, but the different relative proportion of these groups in the two fats allow for the qualitative determination of the shortening mixtures.

3.2. Frequency range 990–950 cm^{-1}

Qualitatively, it is easy to differentiate between the 0% lard and 100% lard in this region. The spectra of fat from cakes with 100% shortening (A) showed a clear band at 975 cm^{-1}, which is known to be due to the C═C─H bending vibration of *trans* double bonds (Firestones & LaBouliere, 1965), whereas the spectra of fat from cakes with 100% lard spectrum (G) has no clear band in this region (Fig. 3). The band in the same region in the IR spectrum is the basis of the AOCS Official Method for determination of *trans* groups (Sedman, Van de Voort, & Ismail, 1997).

As illustrated in Fig. 3, when the ratio of lard to shortening increases, the absorbance value decreases. The spectra of the lard/shortening mixture showed lower absorbance than the 100% shortening spectrum, which indicates that the shortening may contain some *trans* double bond in its composition. According to the manufacturer, this is true because the shortening contain 26% of *trans* acid. Using the data in Table 1, the equation $y = -0.0243x + 0.4071$, (y is the absorbance at 975 cm^{-1} under condition of the test, and x is the weight of lard in lard/shortening mixtures) was obtained for semi-quantitative determination of percent lard in shortening mixtures with R^2 of 0.9830. (Fig. 4).

Table 1
Absorbance values in the FTIR spectra of fat from cakes with 0–60 g lard in shortening for region 990–950 cm^{-1}

Spectrum	Weight of lard (g)	Absorbance (A)
A	0	0.39
B	10	0.35
C	22	0.33
D	30	0.32
E	40	0.28
F	50	0.26
G	60	0.24

3.3. Expanded FTIR calibration model for predicting lard content

An FTIR calibration model was obtained using the two wavelength regions described before, using chemometric PLS for the prediction of lard content in mixture of lard and shortening. Using the calibration data set, a plot of actual weight of lard content in shortening (g/g) vs. FTIR predicted value of lard content in shortening (g/g) ($y = 3.3191x + 0.2462$) was obtained with SE of 1.7520 and R^2 0.9790 (Fig. 5).

After calibrating the model, the validation procedure is carried out to minimize the prediction error and provide an estimate of the overall accuracy of the

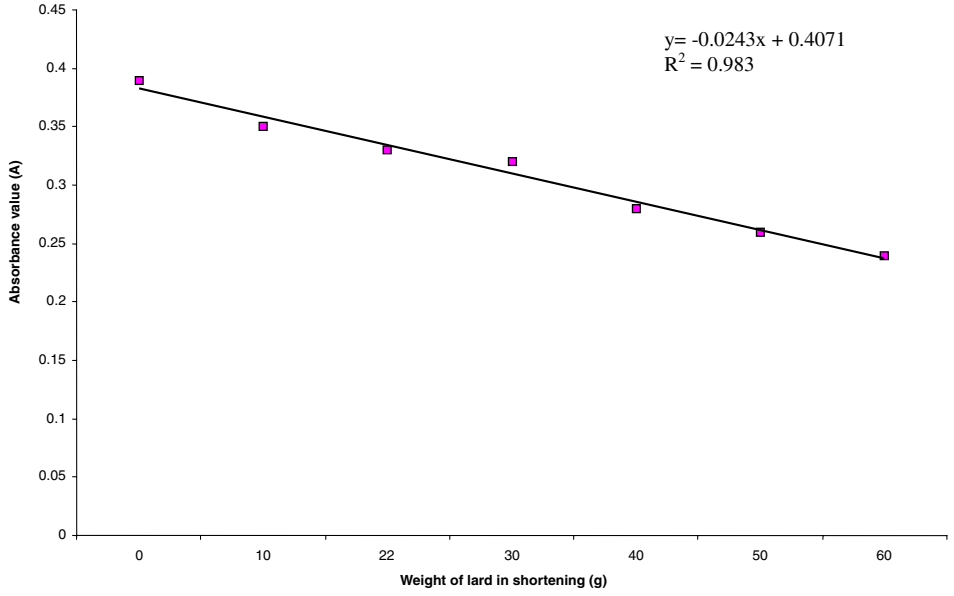

Fig. 4. Frequency value of band 'b' (Fig. 3) in the FTIR spectra of lard/shortening mixtures vs. weight of lard (g).

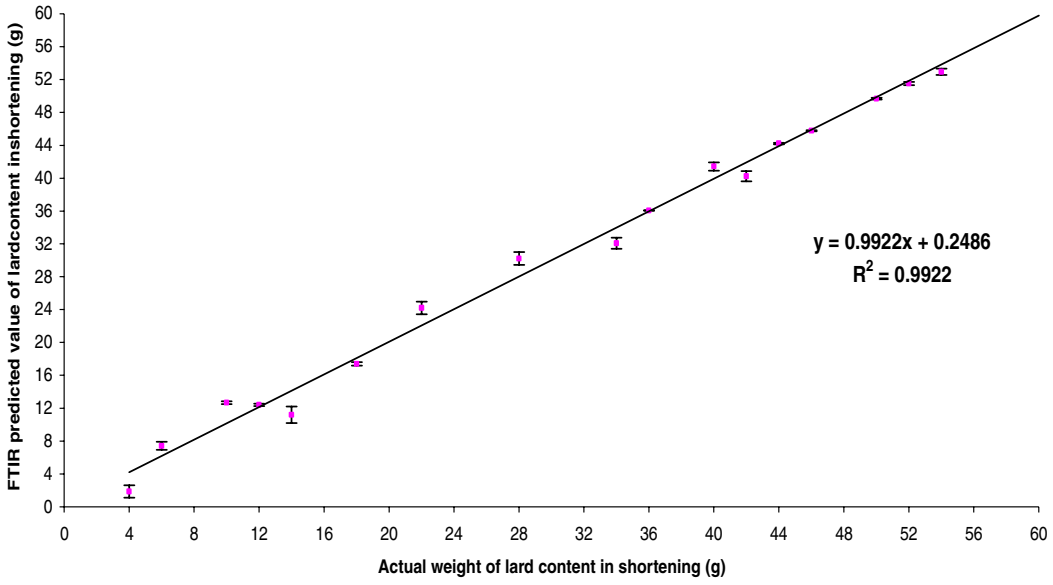

Fig. 5. Plot of actual weight of lard content in shortening (g/g) vs. FTIR predicted value of lard content in shortening (g/g), obtain from calibration plot.

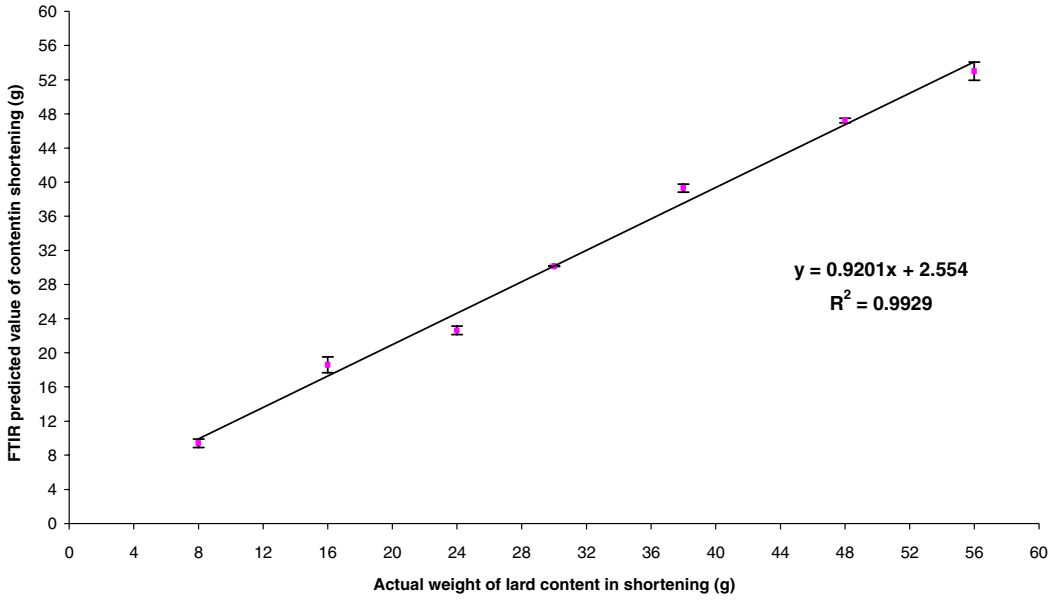

Fig. 6. Plot of actual weight of lard content in shortening (g/g) vs. FTIR predicted value of lard content in shortening (g/g), obtain from validation plot.

predictions. A good linear regression was obtained for the actual value against FTIR predicted value of lard content in shortening, yields an equation; $y = 7.3107x + 2.274$. The SEP obtained was 1.773 and the R^2 was 0.9941 (Fig. 6).

4. Conclusion

It is likely that this FTIR analytical approach would be adaptable to detect and quantify the level of lard adulterated in cake formulation, particularly if the same type of shortening was used in the formulation. It can be said that when the lard is mixed with this shortening in cake formulation, we can check the presence by using 1117–1097 and 990–950 cm^{-1} regions. We have shown here that the combination of ATR with PLS regression made it possible to extract relevant information from MIR spectra of fat from cakes.

In any case of lard adulteration in cakes, when variable lipid sources are used to formulate the products (e.g. other commercial shortening), the calibration

model has to be designed to account for the variation. Before this model can be applied to the cakes available in the market, a database comprising a collection of all shortening spectrum have to be develop first to ensure the accuracy of the prediction.

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