Determination of Lard in Mixture of Body Fats of Mutton and Cow by Fourier Transform Infrared Spectroscopy

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Abstract: Fourier transform infrared (FTIR) spectroscopy provides a simple and rapid means of monitoring lard when blended with mutton and cow body fats. The spectral bands associated with mutton, cow body fats and their lard blends were recorded, interpreted and identified by relating them to those spectroscopically representative pure mutton, cow and lard body fats as references. A qualitative approach is proposed by comparing the pure animal fat with blended ones. Partial least squares (PLS) regression was applied for quantitative determination of the percent of lard in its blend with mutton body fat (MBF) using FTIR spectral data at frequency regions 3010 - 3000, 1220 - 1095 and 968 - 965 cm^{-1} . The equation $y = 1.151x - 0.1882$, coefficient of determination (R^2) = 0.9866 with standard error (SE) of 2.01. For the blend with cow body fat (CBF) frequency regions 1419 - 1414 and 968 - 965 cm^{-1} were used for qualitative and quantitative determination. PLS approach was used to create the equation $y = 0.7239x + 3.1369$ with $R^2 = 0.9749$ and SE = 1.86. The PLS calibration models were cross-validated and the standard deviation of difference (SDD) for repeatability and accuracy and R^2 were computed.

Key words: CBF, FTIR spectroscopy, lard, MBF, PLS

1 Introduction

The Codex Alimentarius (1) specified that all edible animal fats must come from animals fit for human consumption, and stated some analytical identity standards for four products from animal sources. Lard is commonly blended in edible oil/fats product like butter and shortening (2). A number of investigations have been carried out by several research groups to develop analytical methods for detection of animal body fats in ghee or butter (3-6).

Although information has been published concerning animal fats, few analytical methods are available and most are either difficult to perform or time-consuming. DeMan (7) reported that the fatty acid composition of lard was different from cow body fat in C16:1, C18:3, C20:0 and C20:1, and from lamb body fat in C14:0, C16:1, C18:2 and C18:1 n . However, differences in fatty acid composition are too small to be used as indicator (8). In a few cases, methyl ester analysis by gas liquid chromatography (GLC) provides identification of vegetable oil adulteration with animal fats by measure-

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ments of the C17:0 and C17:1 fatty acids. But it should be interpreted with care, because some vegetable oils such as Indian sesame seed oil may contain C17:0 and/or C17:1 acids (9).

Lambelet (3), Lambelet *et al.* (4) and Coni *et al.* (5) had reported on detection of animal body fat in ghee and butter using differential scanning calorimetry (DSC). Since lard contains a high percent of saturated fatty acids in the 2-position of the Triacylglycerols (TAGs) compared to other animal fats, determination of the fatty acids in the 2-position can be used to detect the presence of lard in other animal fats (10). TAG profiles by high-pressure liquid chromatography (HPLC) may indicate adulteration of lard by beef tallow depending on the fatty acids in the 2-position of the TAGs. Foreign fats can be determined in lard by Boemer number method (11) which is based on the difference between melting points of TAGs and the fatty acids, which is large in pure pork fat and small for others such as beef tallow fats. Haryati *et al.* (12), found that differences in TAG group composition in fats are reflected on the DSC thermograms. Guillen and Cabo (13) used the FTIR spectroscopy to characterize edible oil and lard. A recent study by Che Man and Mirghani (14) showed that the FTIR spectroscopy could be used in qualitative and quantitative ways for determining lard in its mixture with other animal fats. The use of FTIR spectroscopy in food analysis has been described by van de Voort *et al.* (15,16) and Che Man and co-researchers (17,18) who had developed FTIR spectroscopic methods for analysis of edible fats and oils. The present study was conducted to elaborate the possibility of detection of lard when mixed with mutton and cow body fats using FTIR spectroscopy.

2 Experimental

2.1 Sample Preparation

Adipose tissues from various parts of slaughtered pig (lard), cow, and mutton body fats (CBF, MBF) were obtained from a local market. The tissues were cut into small pieces, mixed, melted at 90 – 100°C, (19) and strained through a triple-fold muslin cloth. The melted fat was centrifuged at 3000 rpm (Kubota model 2010, Tokyo, Japan) for 20 minutes. The fat layer was decanted into a test tube containing anhydrous sodium sulfate (Na_2SO_4), shaken well and centrifuged again, then decanted through a Whatman filter paper containing

anhydrous Na_2SO_4 . The filtered sample was directly analyzed or kept tightly closed under a nitrogen blanket in the refrigerator.

Lard was mixed with each of other melted animal fat (CBF and MBF) by weight to cover the range of 0 - 25% lard. The pure animal body fats and the mixed samples were analyzed by means of FTIR spectroscopy.

2.2 Instrumentation/Spectral Acquisition

After vigorous shaking for each sample using an Autovortex Mixture SAI (Stuart Scientific, redhill, United Kingdom), a few drops were sandwiched between clean and dry sodium chloride (NaCl) windows. A polytetrafluoroethylene (PTFE) spacer was placed between the windows to give a fixed path-length of 0.1 mm. Cells were fitted in the sample holder in the FTIR spectrophotometer and scanning was carried out for each sample. NaCl windows were rinsed at least three times with pure acetone then dried. Scanning of the samples was carried out using a Perkin-Elmer 1650 series FTIR spectrophotometer (Perkin-Elmer Corporation, Norwalk, CT) equipped with a deuterated triglycine sulfate (DTGS) detector connected to a Perkin-Elmer model 7300 professional computer. Infrared data system (IRDM) software was used during FTIR data collection. FTIR data were collected from 80 scans at resolution of 4 cm^{-1} and strong apodization through the region 4000 to 600 cm^{-1} . All spectra were ratioed against background air spectrum and they were recorded as absorbance values at each data point.

Windows-based software program Spectrum Lite version 1.4 (Perkin-Elmer) was used to obtain the frequency of each band using the "label peaks" command of the software or using the vertical cursor by moving it to find the frequency at the maximum absorbance for the selected band. A PLS statistical approach was developed for mathematical treatments of FTIR data. The assessment of accuracy was based on the smallest standard error (SE) and the highest coefficient of determination (R^2) (20). The "leave-one-out" cross-validation technique was used to validate calibration models, and the accuracy of each model was assessed based on standard error of prediction (SEP) and R^2 . Further evaluation was by comparing the mean difference and standard deviation of differences (SDD) for repeatability and accuracy between the predicted FTIR data and the actual lard content in the samples of animal fat blends.

3 Results and Discussion

3.1 Mutton Body Fat (MBF)

Figure 1 shows the FTIR spectra of both mutton body fat (MBF) and lard. The spectra illustrate the dominant spectral features associated with each of them as for general fats and oils (21), CH stretching absorption in the frequency region $3050 - 2800 \text{ cm}^{-1}$, the carbonyl absorption of the triacylglycerol ester linkage at $1746 - 1744 \text{ cm}^{-1}$ and the bands associated with the fingerprint region $1500 - 1000 \text{ cm}^{-1}$. The differences observed from raw spectra between MBF and lard appears in three frequency regions, $3010 - 3000$, $1120 - 1095$, and $968 - 966 \text{ cm}^{-1}$. These three regions are illustrated in Fig. 1 as a, b and c, respectively.

The difference between spectra of MBF and lard appear in the frequency region $3010 - 3000 \text{ cm}^{-1}$ (a) as shown in Fig. 1 and 2, where lard spectrum has a sharp band at higher frequency (3009 cm^{-1}) than MBF which has a shoulder peak at low frequency (3001 cm^{-1}). Spectra 1%L - 25%L in Fig. 2 represents the blends of 1 - 25 % lard with MBF. Bands in this region (a) are due to the cis double bond stretching vibration (13); accordingly, fats with high proportion of linolenic or linoleic acyl groups show higher frequency for this

Fig. 1 FTIR Spectra of (Lard) Pure Lard and (MBF) Pure MBF. The labeled peaks are absorption bands that are significant in the determination.

band than fats with high proportion of oleic acyl groups. This result agrees with the fatty acid composition for lard and MBF (8), which is high in oleic and lower in linoleic and linolenic compared to lard. At the FTIR frequency region $1120 - 1095 \text{ cm}^{-1}$ lard showed overlap of two peaks having maximum absorption at 1119 and 1100 cm^{-1} , (b) in Fig. 1. This has been found to be related, in an inverse way, to the proportion of sat-

Fig. 2 FTIR Spectra of MBF, (1, 3, 5, 8, 10, 12, 15, 18, 20, and 25% Lard) and Pure Lard Shows Changes in Frequency and Absorbance within the Regions $3010 - 3000$, $1120 - 1095$, and $968 - 966 \text{ cm}^{-1}$ (a, b, and c in Fig. 1) due to % of Lard in the Blend with MBF.

urated *acyl* groups and oleic *acyl* groups, respectively (21). From the above results it could be concluded that both lard and MBF contain saturated and oleic *acyl* groups in their structures in some sort of variation. The MBF spectrum showed a clear band at 966 - 967 cm^{-1} which is known to be due to the C=C-H bending vibration of *trans* double bonds (22,23), while lard spectrum has no clear band (c) in Fig. 1. The band at the same region 975 - 965 cm^{-1} in the IR spectrum is the basis of the AOCS official method for determination of *trans* groups (24).

A quantitative assessment is proposed using the frequency regions (3010 - 3000, 1120 - 1095, and 968 - 965 cm^{-1}) and PLS statistical to assess the variation in frequency and absorbance as a function of the ratio of blending of lard with MBF, which is shown in Fig. 3 and the equation: $Y = 1.151x - 0.1882$ with coefficient of determination (R^2) of 0.9866 and SE of 2.01. Data was cross-validated resulting in R^2 of 0.9769 (Fig. 4) and standard error of prediction (SEP) of 2.23

3.2 Cow Body Fat (CBF)

Figure 5 shows the spectra of both lard and cow body fat CBF. In the frequency region 3008 - 3003 cm^{-1} (a) the CBF has small or shoulder peak while the lard peak is clear and sharp. The second difference is band at frequency of 1418 cm^{-1} (b), which can be attributed to rocking vibrations of CH bonds of *cis*-disubstituted olefins (13). The third difference is for the presence of C=CH- *trans* stretching at frequency 966 cm^{-1} (c),

where there is a sharp peak for the CBF spectrum while no clear one for the lard spectrum.

The bands at 3008 - 3003 cm^{-1} could be used for qualitative analysis to differentiate between pure lard and pure CBF as it shows a sharp peak for lard spectrum while small or a shoulder peak for CBF spectrum. The frequency region 1419 - 1414 cm^{-1} could be used for qualitative determination of lard when blended with CBF covering the range of 00 - 25% (w/w) of lard in CBF. Qualitatively it is easy to differentiate between the FTIR spectra of lard and CBF in Fig. 5, because the last one showed a clear band at frequency 966 cm^{-1} while lard shows no peak at the same frequency due to the content of *trans* fatty acid in CBF (8). In Fig. 6 when the ratio of lard increases, the absorbance at 966 cm^{-1} decreases showing the lower content of *trans* fatty acids in lard than CBF. This is in agreement with the study by Guillen and Cabo (13) that didn't mention any peak at frequency 966 cm^{-1} for lard spectrum. PLS statistical approach was used for quantitative determination of lard when mixed with CBF using the spectral data at the regions 1419 - 1414 and 968 - 965 cm^{-1} . The equation created is $Y = 0.7239x + 3.1369$ with R^2 and SE of 0.9749 and 1.86, respectively (Fig. 7). Figure 8 shows the cross-validation results of $R^2 = 0.96$ and SEP of 2.30

Table 1 shows the statistical results calculated from the cross validation as mean difference (MD) and standard deviations of difference (SDD) for repeatability and accuracy for the determination of both lard blends with

Fig. 3 A Plot of Concentration of Lard (%) in the Blend with MBF versus FTIR-Predicted Concentration of Lard (%) Using PLS and Spectral Data at Frequency Regions 3010-3000, 1120-1095, and 968-965 cm^{-1} for Calibration.

Fig. 4 A Plot of Concentration of Lard (%) in the Blend with MBF versus FTIR-Predicted Concentration of Lard (%) Using PLS and Spectral Data at Frequency Regions 3010-3000, 1120-1095, and 968-965 cm^{-1} for Cross-Validation.

Fig. 5 FTIR Spectra of (Lard) Pure Lard and (CBF) Pure CBF. The labeled peaks are absorption bands that are significant in the determination.

Fig. 6 FTIR Spectra of CBF, (1, 3, 5, 8, 10, 12, 15, 18, 20, and 25% Lard) and Pure Lard Showed Changes in the Absorbance at Frequency Regions 1419 - 1416 and 968 - 966 (b and c in **Fig. 3**).

CBF and MBF.

The use of Fourier Transform Infrared (FTIR) spectroscopy in this study offers a rapid, consistent and reproducible analytical technique that could be used as a quality measure for animal fats in their neat form. Further benefits of using FTIR spectroscopic method is that the tedious time- and chemical - consuming stand-

Fig. 7 A Plot of Concentration of Lard (%) in the Blend with CBF versus FTIR-Predicted Concentration of Lard (%) Using PLS and Spectral Data at Frequency Regions 1419-1416, and 968-965 cm^{-1} for Calibration.

Fig. 8 A Plot of Concentration of Lard (%) in the Blend with CBF versus FTIR-Predicted Concentration of Lard (%) Using PLS and Spectral Data at Frequency Regions 1419-14165, and 968-965 cm^{-1} for Cross-Validation.

ard and chemical methods can be avoided. The study could be extended by using specific sampling regarding - for example - other animals fat, milk fats and vegetable oils. Once the database is established and the model developed, each individual FTIR analysis can be achieved in about 3 - 5 minutes.

Table 1 Repeatability and Accuracy of FTIR Predictions of Lard Content in MBF and CBF Obtained by Cross Validation^a.

Statistics	MBF		CBF	
	Actual	FTIR	Actual	FTIR
MDr	4.06	3.72	4.31	3.29
SDDr	—	-1.24	—	-2.09
MDa	2.49		2.37	
SDDa	1.18		1.14	

^a FTIR, Fourier transform infrared; CBF, cow body fat; MBF, mutton body fat; MD, mean difference; SDD, standard deviation of difference; r, repeatability; a, accuracy.

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