CHAPTER VI

DIFFERENTIATION OF LARD FROM BEEF TALLOW AND CHICKEN FAT BY MEANS OF FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

1 Introduction

In the field of Infrared spectroscopy, there are three main categories depending on the rotatation or vibration corresponding to discrete energy levels (vibrational modes) of the molecules. The first category is far-infrared, approximately 400-10 cm-1, it characterizes by having low energy and may be used for rotational spectroscopy. The mid-infrared is the second class, it is used most for chemical analysis, which corresponds to changes in vibrational energies within molecules approximately 4000-400 cm-1 may be used to study the fundamental vibrations and associated rotational-vibrational structure. Furthermore, the third category is the higher energy near-IR, approximately 13300-4000 cm-1, and it can excite overtone or harmonic vibrations (Mosoba, 1998).

Because of the development of Fourier transform infrared (FT-IR) spectroscopic instrumentation, the application of this technique expanded in food research and particularly has become a powerful analytical tool in the study of edible oils and fats. FT-IR spectroscopy has some advantages over other analytical instruments (including GC, HPLC, and DSC) for example it is rapid, and economic with minimum sample preparation necessary. Infrared spectroscopy is among the most powerful spectroscopic techniques for food analysis since it covers the details on functional group as well as chemical composition that are contained in the infrared spectrum of specific substances (Kumosinski & Farrell, 1993).

Infrared spectroscopy is commonly used in both academic research and industry as a simple and reliable technique for measurement, quality control, and dynamic analysis. Infrared spectroscopy has been utilized for determining a range of adulteration problems in food products such as lard content in cakes and chocolates (Che Man et al., 2005); lard in mixture of animal fats (Che Man & Mirghani, 2001; Jaswir et al., 2003; Syahariza et al., 2005); Rohman & Che Man, 2010; Rohman et al., 2011); adulteration

in jam (Defernez & Wilson, 1995); extra virgin olive oil (Lai et al., 1995); and adulteration in coffee (Paradkar & Irudayaraj, 2002). Vongsvivut et al., (2012) achieved to determine some of fatty acid compositions in micro-encapsulated fish-oil supplements such as omega-3 fatty acids, EPA and DHA using FTIR. *Trans* fatty acids were also successfully detected using FTIR (Sherazi et al., 2009). In addition to its application in the detection of edible fats and oil adulteration, FTIR was also used in the studies of thermal stability of edible oils (Moros et al., 2009; Pinto et al., 2010). The objective of this study was to differentiate lard from other animal fats (chicken, beef fats) as well as their admixtures in different concentrations (lard-beef fat and lard-chicken fat) using FTIR spectroscopy.

2.0 Materials And Methods

2.1 Samples Preparation:

The extraction of fats from the samples was done according to Bligh-Dyer method (1959) with some slight modifications. Accurately, 300 to 400 mg of sample was weighed; then, 4 ml methanol, 2 ml chloroform and 0.4 ml water were added; homogenized and vortexed for 30 sec. After that, 2 ml of chloroform and 2 ml of water were added; vortexed for 30 sec and centrifuged at 5000 × g for 10 min, at room temperature. Using a Pasteur pipette the upper layer (methanol/water) was then removed, and the lower layer (methanol/chloroform) was transferred into a clear tube. Finally, the solvents were removed using a rotary evaporator under reduced pressure, at 60°C. The extracted animal fat, lard, beef fat (BF), chicken fat (CF) and a series of 14 samples containing (0.5 - 10 % w/w) of lard in BF, and CF where analyzed. Samples containing lard were assigned as adulterated; while pure beef tallow, pure chicken fat and pure lard were marked beef tallow (BT), chicken fat (CF) and lard (LD).

2.2 FTIR Spectroscopy Analysis

The obtained fats beef tallow (BT), chicken fat (CF), and lard (LD) were placed- using a Pasteur pipette -in direct contact with attenuated total reflectance (ATR) crystal on a multibounce plate at controlled ambient temperature (25 °C). A Nicolet 6700 FTIR

spectrometer (Thermo Nicolet Corp., Madison, WI) equipped with a detector of deuterated triglycine sulphate (DTGS), a beam splitter of KBr/ Germanium, and connected to software of the OMNIC operating system (Version 7.0 Thermo Nicolet), were used during FTIR data collection. FTIR spectra were recorded from 32 scans at a resolution of 2 cm -1 at 4000–650 cm-1. These spectra were substracted against background air spectrum. After every scan, a new reference air background spectrum was taken. The ATR plate was carefully cleaned with hexane twice followed by acetone and dried with soft tissue before filling in with the next sample. Cleanliness was verified by collecting a background spectrum and compare to the previous one. These spectra were recorded as transmittance values at each data point.

3. Results And Discussion

The representative spectra of fats obtained from the extraction of pork, chicken and beef are demonstrated in Figure16 andAppendix 1.From the first observation by naked eye of the FTIR spectra of lard, beef tallow, and chicken fat, they appear very similar. The spectra describe the different profiles in FA composition present among the studied samples. Yet, it is quite difficult to differentiate lard and other fats and oils by depending just on FA profiles, therefore, FTIR spectra are proposed as a potential means to achieve such differentiation because FTIR spectra, according to (Yap et al., 2007), are considered as ''fingerprint tools''. Meaning that, there are no two fats or oils have the same FTIR spectra, in terms of either, number of peaks or maximum peak intensity (Yap

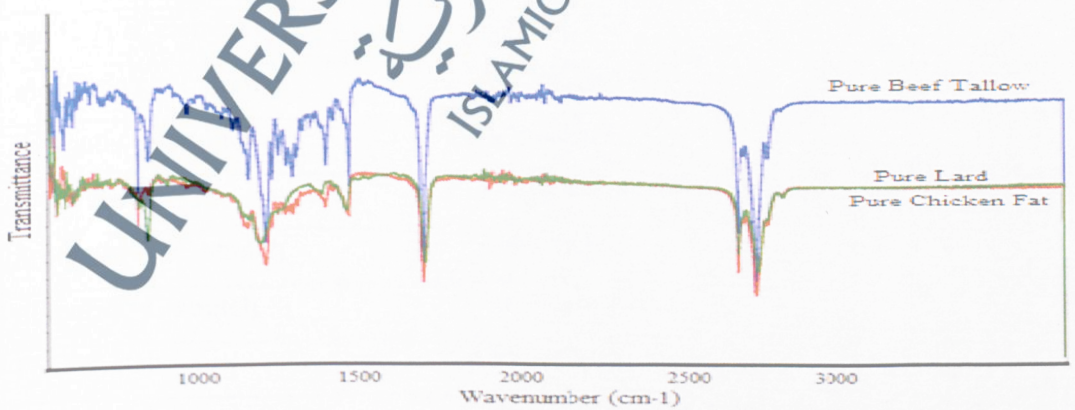

Fig. 16 FTIR spectra of pure beef tallow (BT), chicken fat (CF), and lard (LD)

Table 14. Peak Intensities Of LD, BT, And CF At Certain Frequencies

Transmittance			
Frequency	**BT**	**LD**	**CF**
715.4729	49.6829	40.5509	47.0472
719.3299	57.0556	47.3916	37.2783
887.1093	**72.4006**		
1110.8150	**53.3660**	**40.9464**	**36.8618**
1170.5990	**37.1498**	**29.9837**	
1263.1270	**54.9570**	**44.5645**	
1388.5190	57.0041	46.2387	50.3764
1471.4440	49.9930	44.2870	45.5610
1735.6490	26.6797	22.8406	29.5350
2848.3920	36.6470	27.8632	32.9114
2912.0320	20.0614	20.1473	29.2739
2967.9590		**44.6155**	**44.6155**

Note: Bold numbers refer to the main different frequencies between the samples

Table15. Some Functional Groups And Their Frequencies At IR Spectroscopy

Absorbance	Wavenumber, cm^{-1}
O-H stretch of carboxylic acid	3300-2800 (strong, broad, centers at ~3000)
N-H stretch	3400-3250 (medium intensity, often broad)
sp^2 C-H stretch	3100-3020
sp^3 C-H stretch	2960-2850
aldehyde C-H stretch	2830-2695
aromatic overtones	2000-1600
C=O stretch	1600-1800
aromatic C=C stretch	1600-1585
alkene C=C stretch	1680-1620
C-O stretch	1150-1050
aromatic ring bends	770-730 and 710-690 (for mono-sub'd ring)

(Williamson, 2003)

The FTIR spectra of evaluated samples allowed the identification of certain peaks that help in the characterization, differentiation, and classification of LD, BT, and CF (Van de Voort et al., 2001). The peak intensities of LD, BT, and CF at certain frequencies are listed in Table 14.

In intention of more clarification, FTIR spectrum can be classified in four frequency ranges: 715-900, 950-1480, 1500-1860 and 2500–3050 cm □ ¹. This is based on functional group areas. Table 15 summarizes some functional groups and their frequencies at IR spectra in general.

First Group, Frequency Range 715-900 cm □ ¹:

In this range, it can be observed two peaks exist in all samples with some differences in term of intensity. BT showed very clear peaks in comparison to CF and LD, where, BT exhibit transmittance peaks of 49.6829, and 57.0556; while CF exhibit transmittance peaks of 47.0472 and 37.2783 (peak a), and LD exhibit transmittance peaks of 40.5509 and 47.3916. Furthermore, BT showed an additional small shoulder (peak b) with transmittance of 72.4006 at wavenumber of 887.1093 (cm □ ¹), it might consider as a marker of beef tallow as compared with chicken fat and lard.

Second Group, Frequency Range 950-1480 cm □ ¹:

In this region, BT and LD show more than three peaks, while CF exhibited only three peaks. The longest transmittance peaks are appeared in BTwith intensity of 57.0041, 54.957, and 53.366 at wavenumbers of 1388.519, 1263.127, and 1110.815 cm □ ¹) respectively. On the other hand, LD showed the shortest transmittance peaks in this region with the intensity of 44.287, 40.9464, and 29.9837 at wavenumber of 1471.444, 1110.815, and 1170.599 (cm □ ¹) respectively. In addition of this, CFmade an exception where it showed only three peaks at this part of the spectra. The shortest transmittance peak was 36.8618 at wavenumber of 1110.815 (cm □ ¹) (peak c), while the longest peak was 50.3764 appeared at wavenumber of at 1388.519 (cm □ ¹) (peak d); and these peaks might be considered as markers to characterize chicken fat from other animal fats.

Third Group, Frequency Range 1500-1860 cm □ ¹:

In this part of FTIR spectrum, BT, CF, and LD showed one very clear transmittance peaks at wavenumber of 1735.649 (cm □ ¹) with some differences in term of intensity. For example CFillustrated transmittance peak of 29.535, and BTshowed transmittance of 26.6797, while, LDscored only 22.8406 of transmittance.

Fourth Group, Frequency Range 2500–3050 cm □ ¹:

In this group rang, the three pure samples including BT, CF, and LD have shown two clear transmittance peaks. For the first peak at wavenumber of 2848.392 (cm □ ¹), BT illustrated the highest transmittance of 36.647 and followed by CF with transmittance of 32.9114, while LD showed the lowest transmittance of 27.8632. Moreover, different observation has marked concerning the second peak at frequency of 2912.032 cm □ ¹, where, CF showed the highest transmittance followed by LD and ended by BT with the following transmittance 29.2739, 20.1473, and 20.0614 respectively. In addition, CF and LD have shown an additional overlapping shoulder at wavenumber of 2967.959 (cm □ ¹) with the transmittance of 44.6155.

Figure17 and Figure 18 illustrated the FTIR spectrum of beef tallow (BT) and chicken fat (CF) mixed with 0.5, 1, 2, 3, 4, and 5 % of lard (LD). These figures appear like the pure samples. In other meaning all spectrum kept the similar trends asoriginal spectra. Thus, it is still difficult and impractical to distinguish adulteration of lard in beef tallow (BT) and chicken fat (CF) using FTIR spectra in very small concentrations. In fact, many works have been done in intention of distinguishing lard in mixture with other animal fats using FTIR, for example (Che Man et al., 2001; Guillen & Cabo. 1997; and Rohman, & Che Man. 2009, 2010) but they used more percentage of lard (starting from 5 %).

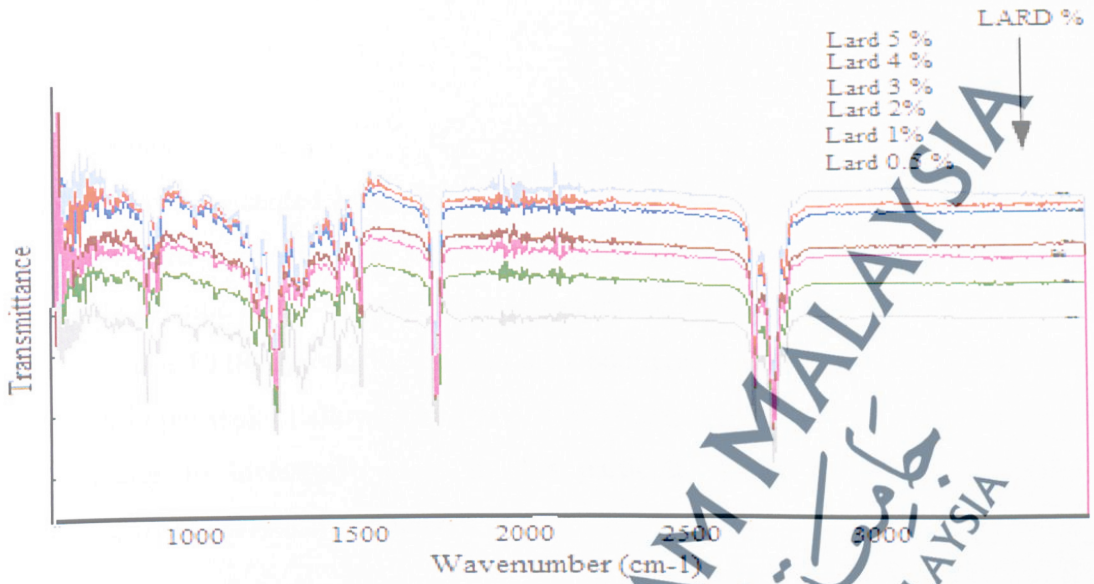

Fig. 17FTIR spectrum of beef tallow (BT) mixed with 0.5, 1, 2, 3, 4, and 5 % of lard (LD).

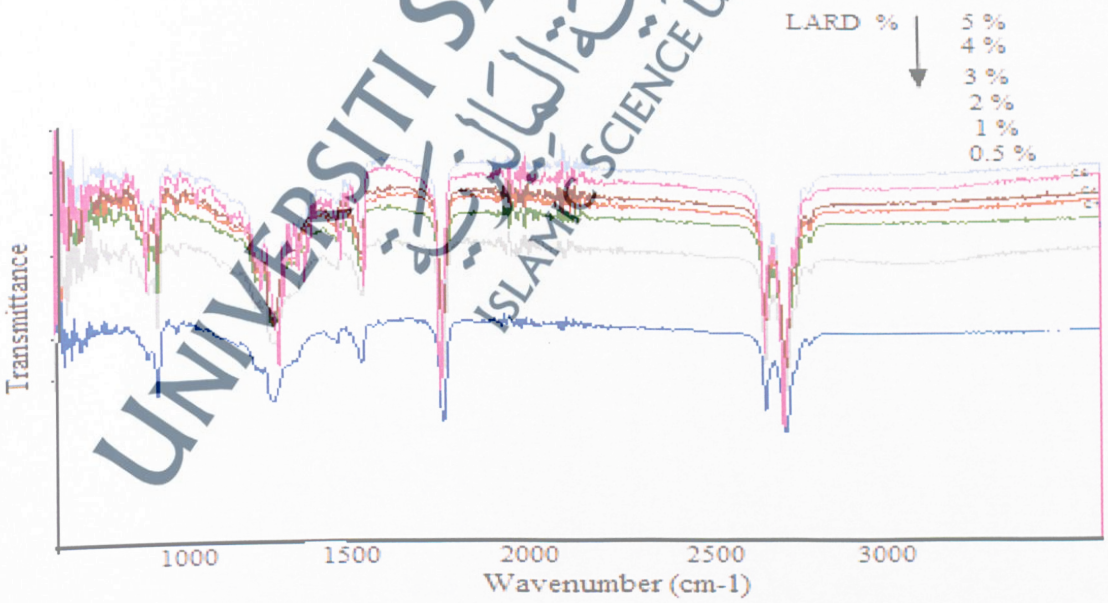

Fig. 18 FTIR spectrum of chicken fat CF mixed with 0.5, 1, 2, 3, 4, and 5 % of lard (LD).

4. Conclusions

From the results presented here, it can be concluded that FTIR spectra can be used to differentiate lard from pure beef tallow and pure chicken. In addition, this technique is rapid and economic compare with chromatographic techniques. However, it is still very difficult to use FTIR spectra for detection of adulterated lard in animal fats in small percentage in particular bellow than 5 % of lard. Thus, It is very much recommended in future studies to investigate more in this particular point for much contribution concerning Halal analytical methods.