

Identification of lard in vegetable oil binary mixtures and commercial food products by FTIR

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RESEARCH ARTICLE

Abstract

Some communities, particularly Muslims and Jews, are very concerned about the authenticity of food products labelled as *Halal*. Fourier transform infrared spectroscopy (FTIR) has been used in this study to detect the adulteration of commercially available food products (frozen French fries) and vegetable oils (corn oil, sunflower oil, palm oil and olive oil) by lard. Lard-vegetable oil binary mixtures ranging from 1 to 20% (1, 5, 10 and 20% lard) were prepared and FTIR was able to detect levels of lard as low as 1% in all mixtures. The FTIR spectral regions at 1,405-1,365, 1,260-1,198, 935-910, 877-857 and 857-833/cm provided the basis for identification of lard either in lard-vegetable oil binary blends or in pre-frying vegetable oils extracted from frozen French fries. FTIR can be used to detect adulteration of processed food products by lard.

Keywords: adulteration, processed food products, frozen French fries, *Halal* authenticity

1. Introduction

Authenticity is an important criterion for food safety and quality. Foods certified as *Halal* are readily acceptable by Muslim and orthodox Jewish consumers who prohibit the consumption of pork, lard, and their derivatives in any product (Regenstein *et al.*, 2003). This acceptance is due to the wholesomeness concept of *Halal*, which covers not only the *Shariah* requirements but also the hygienic and safety aspects of food. Moreover, it protects the consumer against fraud and adulteration (Ebbeca and Thomsen, 1991). Currently, the *Halal* food market makes up to 12% of the overall trade in agricultural food products. It is projected that by the year 2025, Muslims would account for 30% of the world's population (Farouk *et al.*, 2006).

Vegetable oils can be blended with lard to yield products, such as margarine, shortening, and some other specialty foods (Gillies, 1974). The edible fats and oils can be differentiated from each other by determining their particular constituents, like derivatives of fatty acid methyl esters and triacylglycerols using gas chromatography and

liquid chromatography, respectively (Mansor *et al.*, 2011; Marikkar *et al.*, 2005; Rohman *et al.*, 2012; Spencer, 1976). However, these methods are lengthy and time-consuming.

Fourier transform infrared (FTIR) spectroscopy with chemometrics of Partial Least Square has been developed to detect the adulteration of fats and vegetable oils (Che-Man *et al.*, 2011a, 2014; Gurdeniz and Ozen, 2009; Lai *et al.*, 1995; Manaf *et al.*, 2007; Mansor *et al.*, 2011; Marikkar, 2004; Mata *et al.*, 2012; Ozen and Mouer, 2002; Rohman and Che-Man, 2009, 2010; Rohman *et al.*, 2011; Rusak *et al.*, 2003; Quiñones-Islas *et al.*, 2013). FTIR and discriminant analysis has been successfully employed on real food samples likes biscuits to discriminate lard, commercial vegetable oils and animal fats into different groups (Che-Man *et al.*, 2011b). The peaks and shoulders in the FTIR spectra can be assigned to different functional groups which serve as a fingerprint tool of specific fats or oils (Guillén and Cabo, 1997a,b). FTIR is a useful and simple technique for detecting lard in body fats of chicken, lamb and cow (Che-Man and Mirghani, 2001). This technique is also able to detect lard adulteration in cake formulations (Syahariza

et al., 2005), chocolate and chocolate products (Che-Man *et al.*, 2005).

The detection of pork, lard and their derivatives in food products has a great challenge to researchers, food processors, food regulators and food industry due to lack of reliable methods of detection, particularly in processed foods. The current study was conducted to investigate the possibility of using FTIR for the detection of lard mixed with vegetable oils and in some of commercial food products pre-fried in vegetable oils. To date, no report on the detection and identification of lard by FTIR in commercially processed food products which were pre-fried in vegetable oils has been published. Moreover, using FTIR, the detection of lard was also carried out in lard-vegetable oil binary mixtures.

2. Materials and methods

Vegetable oils, namely corn oil, palm oil, olive oil and sunflower oil were purchased from local supermarkets in Riyadh, Saudi Arabia. Lard-vegetable oil blends were prepared according to a previously reported method with slight modifications (Che-Man *et al.*, 2005). Lard was melted at its melting point and then mixed with the vegetable oils at different proportions to yield lard-vegetable oil binary mixtures containing 1 to 20% (w/w) lard. Pure lard and pure vegetable oils were used as positive controls. The samples were coded as: A (pure lard), B to E (lard-vegetable oil binary mixtures containing 20, 10, 5, and 1% lard, respectively) and F (pure vegetable oil). The samples were then kept in refrigerator (~5 °C) until used for FTIR analysis.

Five imported frozen French fries samples (coded as FF1, FF2, FF3, FF4 and FF5) were purchased from the local supermarkets in Riyadh, Saudi Arabia. These French fries were already pre-fried in different vegetable oils by the manufacturer. The pre-frying oils of frozen French fries as mentioned on their labels are listed in Table 1 and were extracted with hexane in our lab for further analysis by FTIR.

Table 1. The coded frozen French fries samples and the set of information given on their labels.

Coded sample	Product description
FF1	Frozen French fries pre-fried in palm oil
FF2	Frozen French fries pre-fried in palm oil
FF3	Frozen French fries pre-fried in soybean and canola oils
FF4	Frozen French fries pre-fried in sunflower, cotton seed, soybean and canola oils
FF5	Frozen French fries pre-fried in sunflower, cotton seed, soybean, palm, canola and partially hydrogenated soybean oils

FTIR analysis

A Vertex 70 FTIR spectrometer (Bruker Optics, Ettlingen, Germany) was used to obtain the spectra of lard, oil binary mixtures and oils extracted from frozen French fries, under investigation. The samples were placed, using a Pasteur pipette, directly in contact with a horizontal attenuated total reflectance (ATR) element (ZnSe crystal) at room temperature (~25 °C). FTIR data were recorded at mid-infrared region (4,000-500/cm), with 32 scans at a standard resolution of 4/cm. All spectra were automatically evaluated against a background air spectrum and recorded as absorbance values at each data point in duplicate using OPUS software (Bruker Optics).

3. Results and discussion

Lard-vegetable oil binary mixtures

The FTIR spectra of lard-vegetable oil binary mixtures are shown in Figures 1-5. There were 10 possible regions of interest (a, a-1, a-2, a-3, b, c, d, d-1, e, and e-1) along the mid-infrared region 4,000-500/cm for each individual vegetable oil and their binary mixtures with lard (Figures 1-5 and Table 2).

However, there were only five spectral regions (a-2, b, d-1, e, and e-1), which could discriminate lard from the vegetable oils (Figure 6 and 7 and Table 2). The FTIR spectra of expanded region (a-2), between 1,405 and 1,365/cm showed one sharp peak at 1,378/cm for lard and palm oil, compared to two peaks, one smaller at around 1,398 and one bigger at 1,377/cm for corn, sunflower and olive oils (Figure 6A and Table 2). The FTIR spectra of expanded region (b), between 1,260-1,198/cm, illustrated two sharp peaks, one bigger at 1,239 and one smaller at 1,220/cm for lard, whereas only one clear sharp peak at 1,236/cm for all vegetable oils was noted (Figure 6B and Table 2).

The spectra for all vegetable oils were identical. The FTIR spectra of expanded region (d-1), between 935-910/cm indicated very broad or no peak at 914/cm for lard, palm oil and olive oil, compared to one clear sharp peak for corn and sunflower oils (Figure 7A and Table 2). The FTIR spectra of expanded region (e) between 877-857/cm revealed one peak at 865/cm for lard and no peak for all vegetable oils (Figure 7B and Table 2). The spectra for all vegetable oils were identical in this region. Lard, palm and olive oils showed no peak in expanded region (e-1) of FTIR spectra between 857-833/cm, but there was one clear peak at 845/cm for corn and sunflower oils (Figure 7C and Table 2).

There were very small differences among the FTIR spectra of lard and lard-vegetable oil binary mixtures, because all vegetable oils are primarily composed of 90-95% triacylglycerols. The differences among oils are due to

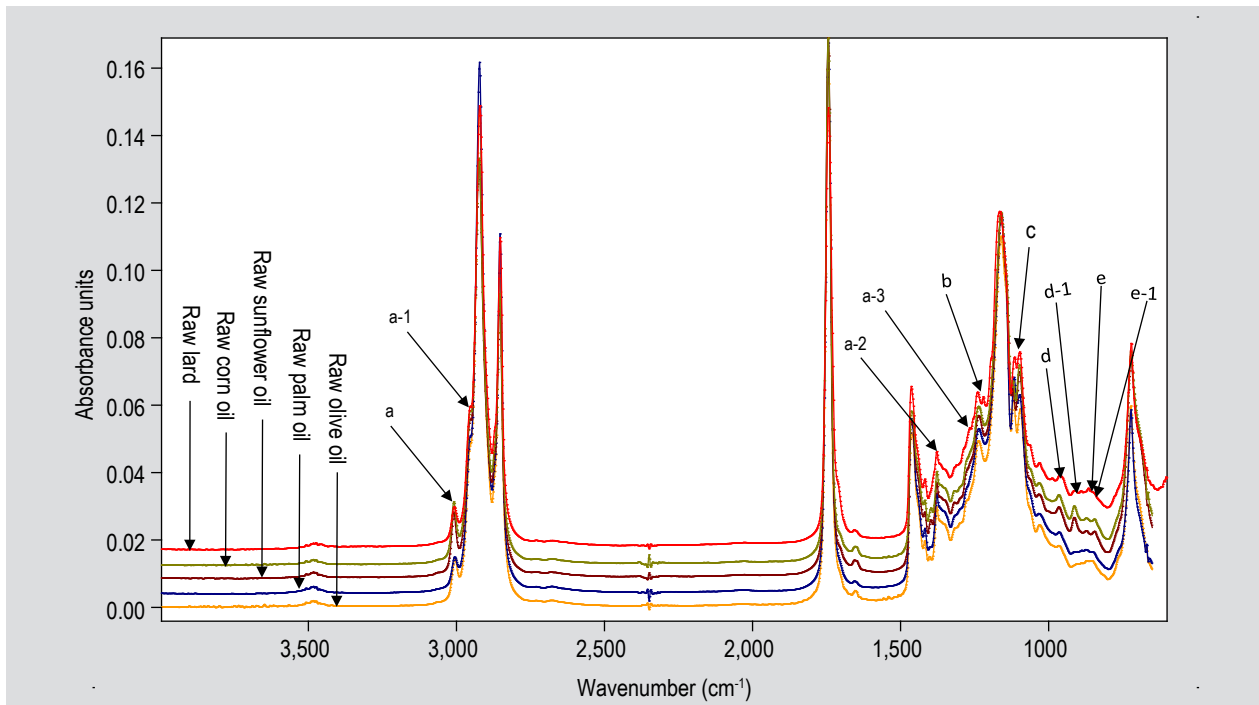


Figure 1. FTIR spectra of lard and raw vegetable oils at mid-infrared region of 4,000-500/cm. The regions of interest are 'a-2', 'b', 'd-1', 'e' and 'e-1'.

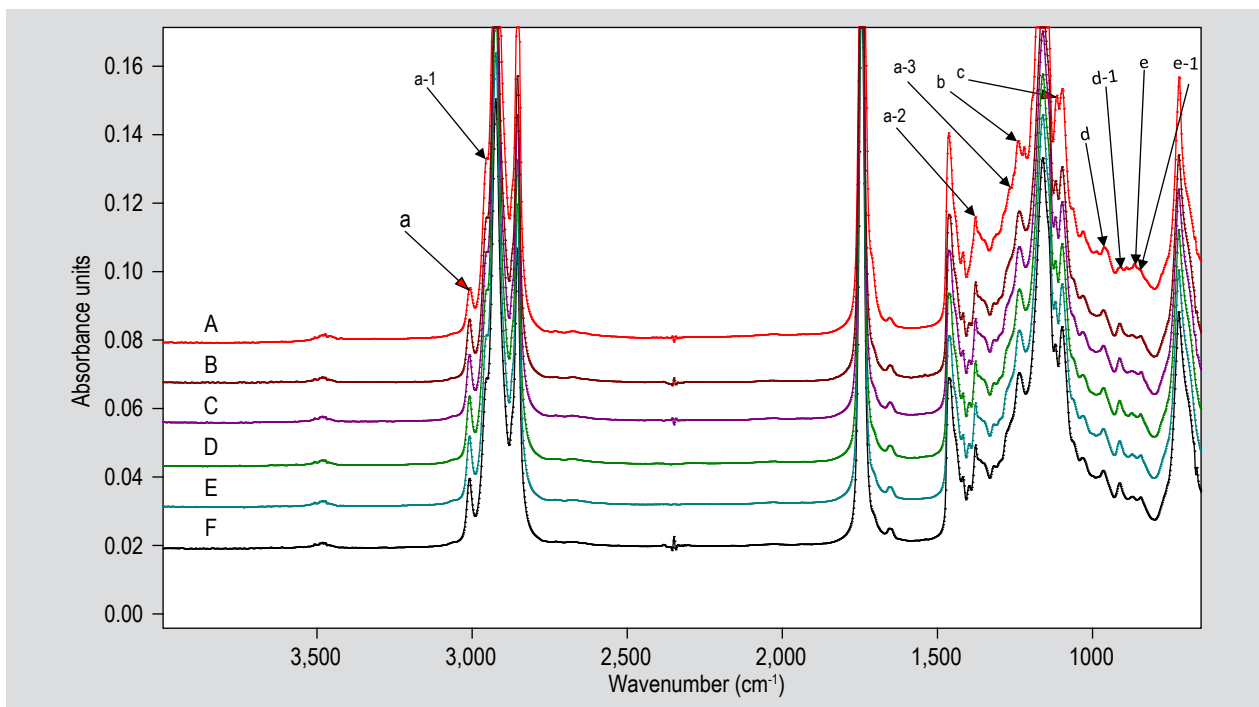


Figure 2. FTIR spectra of (A) pure lard, (B)-(E) lard/corn oil blends, (F) pure corn oil. The percentages of the lard in blends are (B) 20, (C) 10, (D) 5, (E) 1%. The regions of interest are 'a-2', 'b', 'd-1', 'e' and 'e-1'.

variations in fatty acid composition, caused by plant variety, processing and environmental conditions (Ozen and Mouer, 2002). The FTIR with chemometrics approach was used for the analysis of extra virgin olive oil adulterated with

vegetable oils (Gurdeniz and Ozen, 2009; Lerma-García *et al.*, 2010; Mata *et al.*, 2012; Rohman and Che-Man, 2010, 2011, 2012). All spectra looked very similar with minor differences due to their different glycerol composition

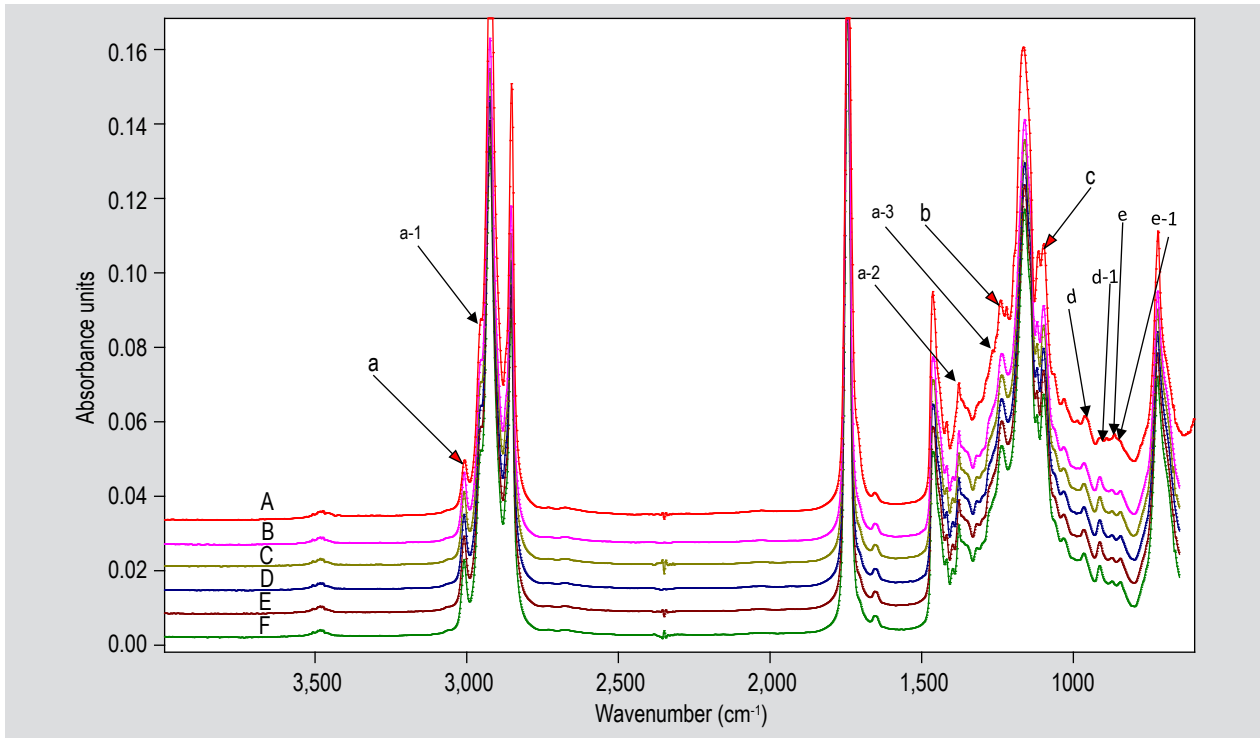


Figure 3. FTIR spectra of (A) pure lard, (B)-(E) lard/sunflower oil blends, (F) pure sunflower oil. The percentages of the lard in blends are (B) 20, (C) 10, (D) 5, (E) 1%. The regions of interest are 'a-2', 'b', 'd-1', 'e' and 'e-1'.

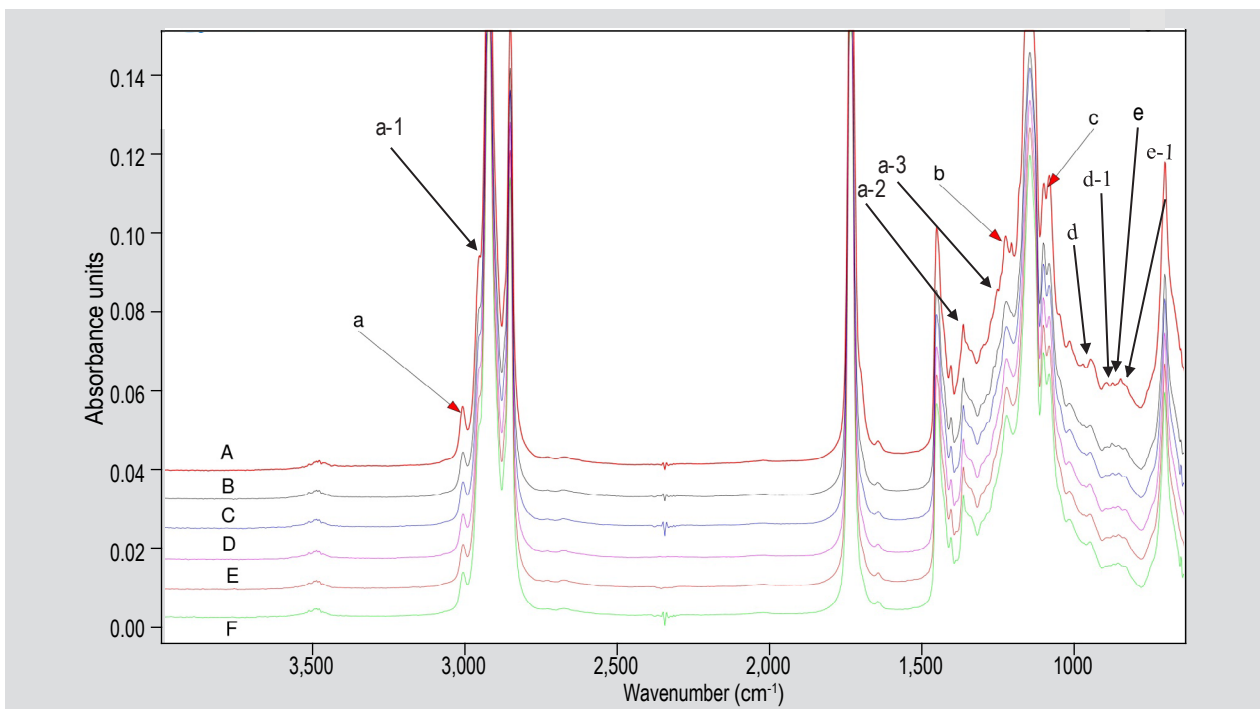


Figure 4. FTIR spectra of (A) pure lard, (B)-(E) lard/palm oil blends, (F) pure palm oil. The percentages of the lard in blends are (B) 20, (C) 10, (D) 5, (E) 1%. The regions of interest are 'a-2', 'b', 'd-1', 'e' and 'e-1'.

in terms of small band shifts and of small changes in the relative peak intensity at certain frequency regions especially at 3,007 and 1,117/cm. However, the frequency

regions for classification and quantification were 1,200-900 and 3,020-3,000/cm (Rohman and Che-Man, 2012).

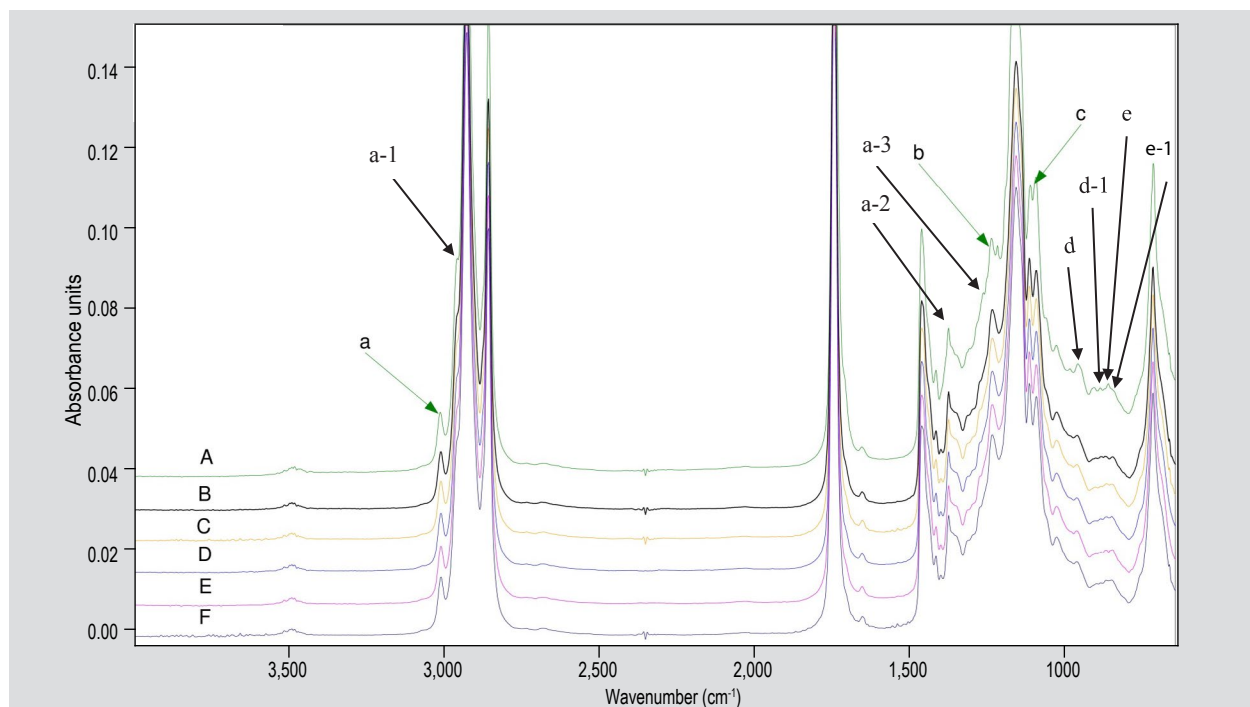


Figure 5. FTIR spectra of (A) pure lard, (B)-(E) lard/olive oil blends, (F) pure olive oil. The percentages of the lard in blends are (B) 20, (C) 10, (D) 5, (E) 1%. The regions of interest are 'a-2', 'b', 'd-1', 'e' and 'e-1'.

Table 2. The FTIR possible differentiating frequencies between lard and some vegetable oils at mid-infrared region of 4,000-500/cm, extracted from Figures 1-7.

Sample	Region of interest (per centimeter)									
	a	a-1	a-2	a-3	b	c	d	d-1	e	e-1
	3,008-3,005	2,970-2,945	1,405-1,365	1,275-1,260	1,260-1,198	1,137-1,076	990-950	935-910	877-857	857-833
Lard	one sharp peak: 3,007	one weak shoulder peak: 2,953	one sharp peak: 1,378	very weak broad peak/ or no peak: 1,265	one bigger peak: 1,239; one smaller peak: 1,220	twin overlapping sharp peaks smaller 1,115: bigger 1,097	very broad peak/ or no peak: 963	very broad peak/ or no peak: 914	one peak: 865	no peak
Corn oil	one sharp peak: 3,009	one weak shoulder peak: 2,953	one small peak: 1,398; one bigger sharp peak: 1,377	no peak	one clear sharp peak: 1,236	small peak: 1,120; clear sharp peak: 1,098	one broad peak: 966	one clear sharp peak: 914	no peak	one clear peak: 845
Sunflower oil	one sharp peak: 3,009	one weak shoulder peak: 2,953	one small clear peak: 1,398; one bigger sharp peak: 1,377: (similar to corn oil)	no peak	one clear sharp peak: 1,236	small sharp peak: 1,120; one sharp peak: 1,098	one broad peak: 966	one clear sharp peak: 914: (similar to corn oil)	no peak	one clear peak: 845
Palm oil	one sharp peak: 3,007	one weak shoulder peak: 2,953	one sharp peak: 1,377: (similar to lard)	no peak	one clear sharp peak: 1,236	one big sharp peak: 1,117; sharp peak 1,098	one broad peak: 964	no peak: (similar to lard)	no peak	no peak
Olive oil	one sharp peak: 3,005	one weak shoulder peak: 2,953	one small peak: 1,402; one bigger sharp peak: 1,377	no peak	one clear sharp peak: 1,237	two clear sharp peaks: 1,119 and 1,096	no peak	no peak	no peak	no peak

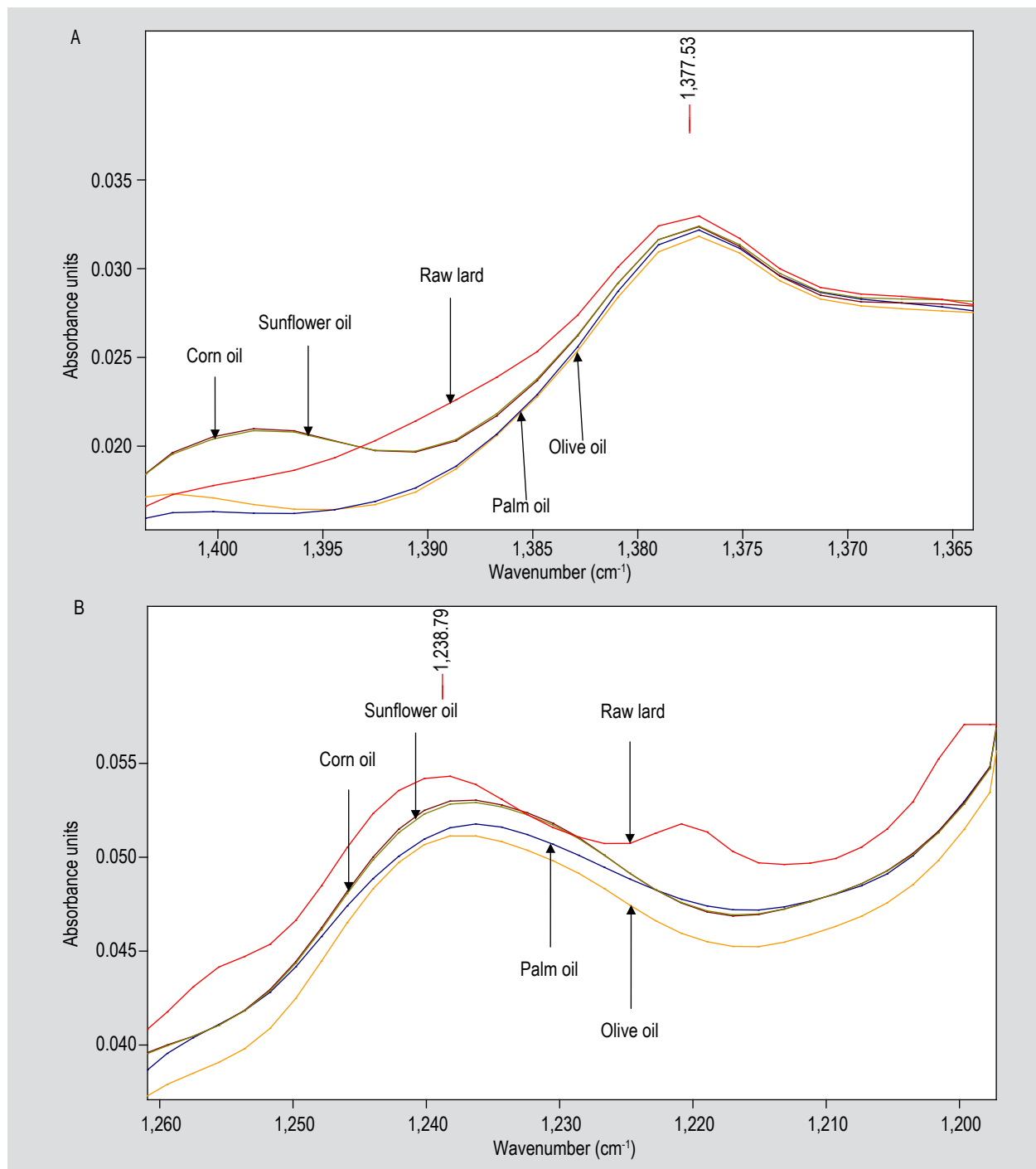


Figure 6. (A) FTIR spectra of expanded region 'a-2' (1,405-1,365/cm); and (B) region 'b' (1,260-1,198/cm) of Figure 1.

According to Vlachos *et al.* (2006) and Yap *et al.* (2007), the fingerprint of any particular fat or oil by FTIR means the sum of peaks and shoulders, their individual intensities, and the exact frequencies of maximum absorptions. The regions of interest in FTIR spectra can pinpoint any characteristic feature peculiar to any fat or oil. The bands (peaks and shoulders) were well-resolved and can be assigned to different functional groups as described by Guillén and Cabo (1998), Bendini *et al.* (2007) and Lerma-García *et al.* (2010).

Che-Man *et al.* (2014) reported that the spectra of palm oil and lard revealed differences at two frequency regions (3,006/cm, and 1,120-1,095/cm). The band at 3,006/cm is related to the stretching vibration of *cis* olefinic double bond (Guillén and Cabo, 1998). The sharper band at 3,006/cm in lard spectrum, compared to palm oil spectrum, was due to a higher content of linolenic acyl groups in lard (double that of palm oil) (Che-Man *et al.*, 2014). In our study, the peak area and peak height gradually increased

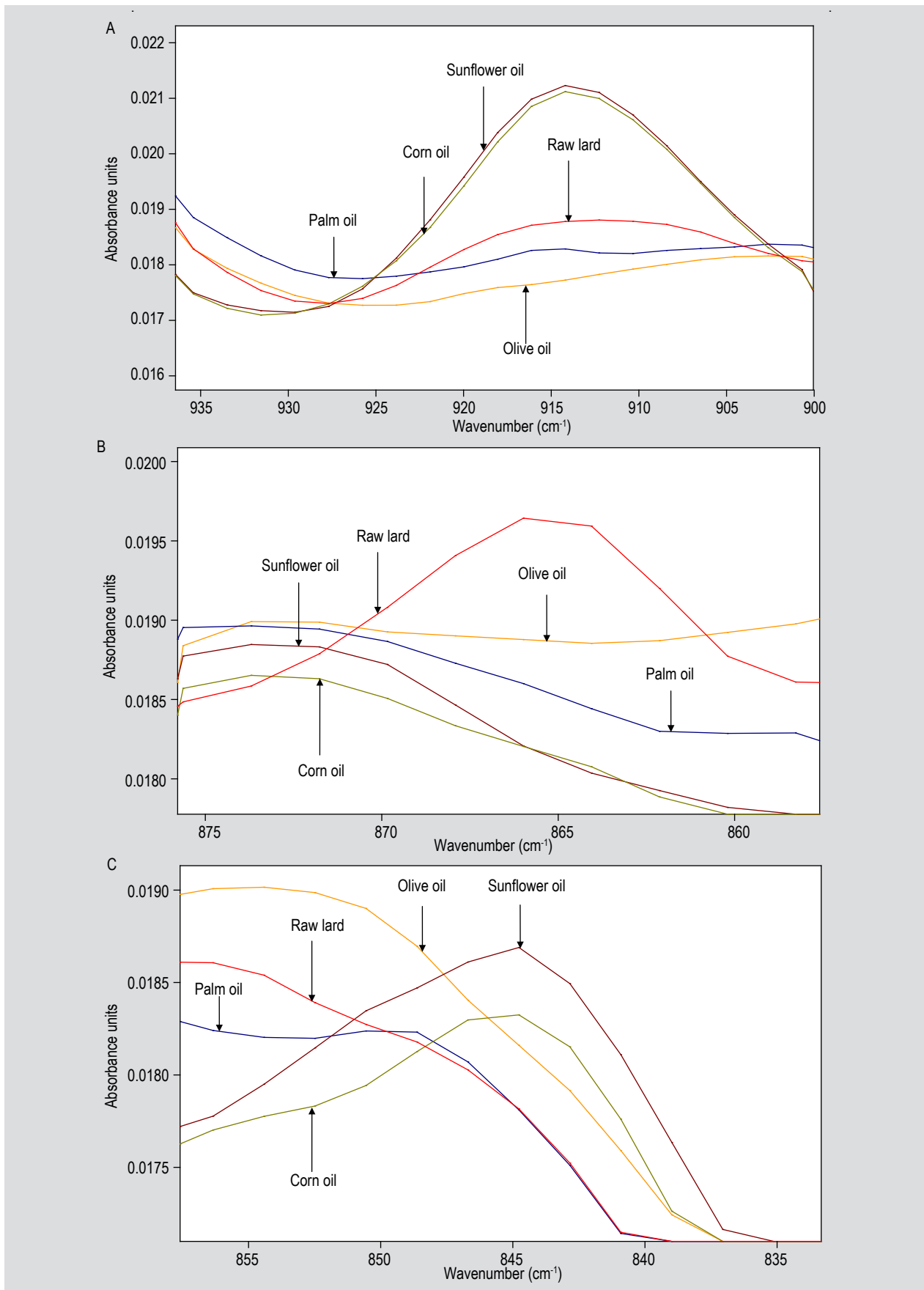


Figure 7. (A) FTIR spectra of expanded region 'd-1' (935-910/cm); (B) region 'e' (877-857/cm) and (C) region 'e-1' (857-833/cm) of Figure 1.

as the percentage of lard in palm oil was increased (Figure 4). The lard was detected up to 1% of its concentration in all of the lard-vegetable oil binary mixtures (Figures 2-5). Marikkar *et al.* (2005) stated that lard in fat mixtures and some vegetable oils can easily be distinguished up to 2% using liquid chromatography coupled with multivariate analysis. It was also possible to detect the lard as low as 1% in refined, bleached and deodorised palm olein using a surface acoustic wave sensing electronic nose (Che-Man *et al.*, 2005). Vlachos *et al.* (2006) found the detection limit of olive oil adulteration as high as 9% using FTIR when the adulterant was sesame seed oil or corn oil, while it was lower (6%) if the adulterant was soybean or sunflower oil. Using FTIR with chemometrics, the detection limit of adulterants like corn-sunflower binary mixture, cottonseed and rapeseed oils in extra-virgin olive oil was found to be as low as 5% (Gurdeniz and Ozen, 2009). Quiñones-Islas *et al.* (2013) detected the level of adulterant (sunflower oil, canola oil, and soybean oil) in avocado oil, as low as 2% using mid-FTIR spectroscopy and multivariate analysis (partial least squares). According to Bingning *et al.* (2015), the determination limit of soybean oil, adulterated in walnut oil, was 10% by FTIR spectroscopy, while fluorescence spectroscopy had a high sensitivity with a determination limit of less than 5%.

In region 'c' at 1,137-1,076 (Table 2), both lard and palm oil spectra showed an overlap of unequal height of peaks at 1,115 and 1,097/cm; similar peaks were also found by Che-Man *et al.* (2014) at 1,119 and 1,100/cm with equal height in case of lard, compared to unequal height in palm

oil spectrum. According to Jaswir *et al.* (2003), the peak heights were inversely correlated with a proportion of saturated acyl groups and oleic acyl groups.

Che-Man and Rohman (2011) demonstrated quite identical FTIR spectra of lard, corn oil, canola oil, sunflower oil and extra virgin olive oil at mid-infrared region (4,000-650/cm). The peaks were at frequencies of 3,007, 2,953, 2,922, 2,853, 1,743, 1,465, 1,377, 1,237, 1,160, 1,117, 1,098, and 721/cm, however, it was hard to discriminate lard from the vegetable oils by the naked eye. According to Rohman and Che-Man (2011), FTIR can be a potential technique for the simultaneous analysis of extra virgin olive oil and virgin coconut oil in the mixtures at the combined frequency regions of 3,050-3,000, 1,660-1,650, and 1,200-900/cm. In another study, Che-Man *et al.* (2011b) compared FTIR spectra of lard with 16 edible fats and oils at mid-infrared region (4,000-650/cm) and found the main frequency regions of 2,852.8, followed by 2,922 and 1,467/cm.

Frozen French fries samples

The results of FTIR spectroscopy of lard and oil/oils extracted from five frozen French fries samples are shown in Figures 8 and 9 and Table 3. There were 8 possible common regions among lard and extracted pre-frying vegetable oils, but only two spectral regions; (b), between 1,260-1,198/cm (Figure 9A), and (d-1), between 935-910/cm (Figure 9B) were of interest with regard to discriminating lard from pre-frying vegetable oils.

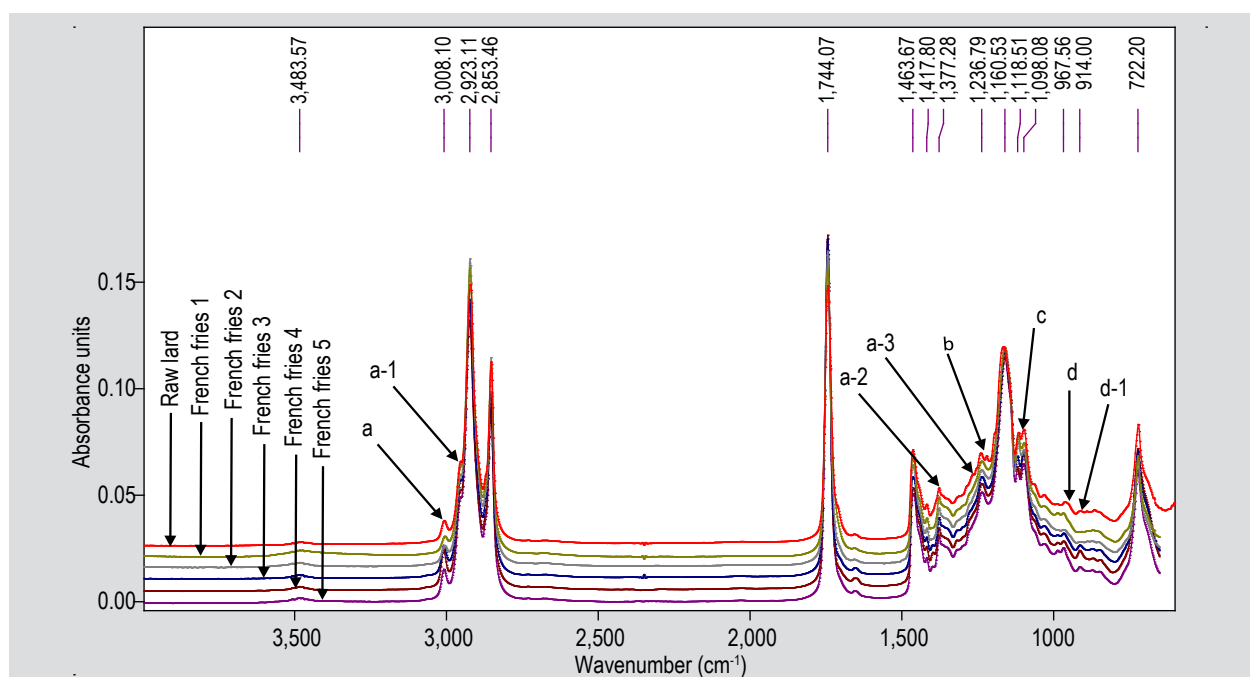


Figure 8. FTIR spectra of lard and commercial foods (frozen French fries samples) at mid-infrared region of 4,000-500/cm. The regions of interest are 'b' and 'd-1'.

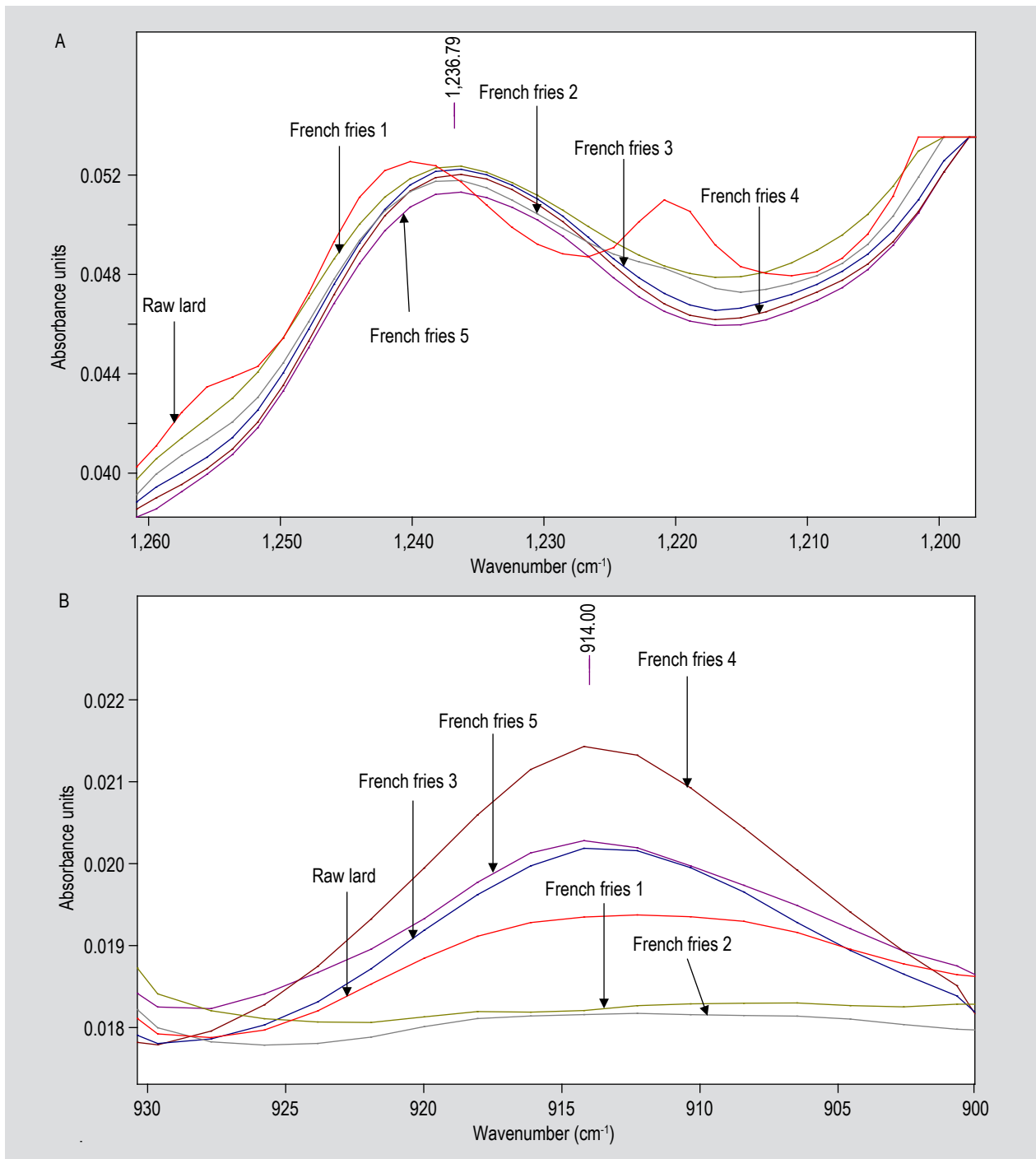


Figure 9. (A) FTIR spectra of expanded region 'b' (1,260-1,198/cm); and (B) region 'd-1' (935-910/cm) in Figure 8.

By observation with the naked eye it is very hard to distinguish lard from edible oils. However, a detailed exploration of the fingerprint region, particularly at wavenumber of 1,400-800/cm, revealed that there were visual variances at absorption peaks of 1,237/cm (region b) and 914/cm (region d-1). The FTIR spectra of expanded region (b) showed two sharp peaks, one bigger at 1,239 and one smaller at 1,220/cm, but only one sharp peak at

1,237/cm for pre-frying vegetable oils extracted from the five French fries samples (Figure 9A and Table 3).

The FTIR spectra of expanded region (d-1), between 935-910/cm, illustrated a very broad peak or no peak at 914/cm for lard and extracted oils from the two French fries samples, coded FF1 and FF2, but with one small peak at 914/cm for extracted oils from the three French fries samples coded FF3, FF4, and FF5 (Figure 9B and Table 3).

Table 3. FTIR possible differentiating frequencies between lard and commercial foods (frozen French fries samples) at mid-infrared region of 4,000-500/cm, extracted from Figure 8 and 9.

Sample	Region of interest (per cm)							
	a 3,008-3,005	a-1 2,970-2,945	a-2 1,405-1,365	a-3 1,275-1,260	b 1,260-1,198	c 1,137-1,076	d 990-950	d-1 935-910
Lard	one sharp peak: 3,007	one weak shoulder peak: 2,953	one sharp peak: 1,378	very weak broad peak/or no peak: 1,265	one bigger peak: 1,239; one smaller peak: 1,220	twin overlapping sharp peaks: smaller 1,115; bigger 1,097	very broad peak/or no peak: 963	very broad peak/or no peak: 914
FF1	one sharp peak: 3,007	one weak shoulder peak: 2,955	one sharp peak: 1,378	no peak	one sharp peak: 1,237	twin equal sharp peaks: 1,116 and 1,097	two very small peaks: 969 and 987	no peak
FF2	one sharp peak: 3,007	one weak shoulder peak: 2,955	one sharp peak: 1,378	no peak	one sharp peak: 1,237	twin equal sharp peaks: 1,116 and 1,097	very weak broad peak: 965	no peak: (similar to FF1)
FF3	one sharp peak: 3,007	one weak shoulder peak: 2,954	one sharp peak: 1,378; one broad peak: 1,400	no peak	one sharp peak: 1,237	twin equal sharp peaks: 1,118 and 1,097	very weak broad peak: 965	one small peak: 914
FF4	one sharp peak: 3,009	one weak shoulder peak: 2,954	one sharp peak: 1,378; one small peak: 1,399	no peak	one sharp peak: 1,237	twin equal sharp peaks: 1,119 and 1,097	very broad peak: 966	one sharp peak: 914
FF5	one sharp peak: 3,008	one weak shoulder peak: 2,954	one sharp peak: 1,377	no peak	one sharp peak: 1,237	twin equal sharp peaks: 1,119 and 1,097	one broad peak: 968	one small peak: 914 (similar to FF3)

These differences in peaks can be exploited for the detection of lard in vegetable oils.

The different peaks in the spectral region (b) between 1,260-1,198/cm (Figure 9A and Table 3) for lard and edible oils extracted from French fries samples, can be attributed to their different chemical components. The specific peaks at 1,237 and 1,220/cm differentiate lard from pre-frying vegetable oils. These results are in accordance with the findings of Che-Man and Rohman (2011) who described that frequencies of 1,117 and 1,236/cm influenced lard differentiation during Principal Component Analysis (PCA) for sunflower, canola, corn, extra virgin olive oil, and soybean oils. Moreover, these data also support the spectral bands in the same fingerprint region of palm oil and sunflower oil given in Table 2.

The variations in the intensities of spectral bands of various oils and fats are associated with the composition of molecular bonds absorbing at those regions (Gurdeniz and Ozen, 2009). There was a notable difference between lard and pre-frying oils extracted from FF3, FF4 and FF5 at 914/cm resulting from bending vibration of *cis* disubstituted olefinic groups (Van de Voort, 1995), whereas FF1 and FF2

samples, which used palm oil as the pre-frying oil, showed the similar spectra as of lard at the given band. Gurdeniz and Ozen (2009) found that corn-sunflower oil binary mixture, cottonseed and rapeseed oil as adulterant in olive oil exhibited some intensity in the band around 913-914/cm, whereas, the olive oil showed very low or no intensity at this wavenumber. Similar results were reported by Che-Man *et al.* (2011a) who could not find any peak for lard at 914/cm in FTIR spectra. However, Yang and Irudayaraj (2000) demonstrated that the spectra of lard and soybean oil obtained by FTIR photoacoustic spectroscopy (FTIR-PAS) and FTIR attenuated total reflectance spectroscopy (FTIR-ATR) techniques, were similar due to similar functional groups. These findings are not in agreement with our results and it might be due to the different method of sample preparation for the analysis by FTIR-PAS and FTIR-ATS. The composition of the fats and oils determines the exact position of the band and it yields shifts when the ratio of the fatty acid changes (Vlachos *et al.*, 2006).

4. Conclusions

The FTIR Spectroscopy was successfully applied for the differentiation and identification of lard in lard-vegetable oil binary blends and in commercial food products, like frozen French fries. Specific FTIR spectral regions proved to be very helpful for assessing adulteration. FTIR might be a very useful technique in detecting lard adulteration in processed foods in a simple and fast way to ensure the supply of *Halal* foods to the Muslim and orthodox Jewish community.

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