Rohman and Che Man [11]. A total of sixteen different fats were obtained.

A. Fat Identification in Pure Samples

The graph shows the average spectrum of three replicates of pig, chicken, beef and lamb fats. The four fats were each subjected to four types of processes namely oven, baking, boiling, and friying. A total of sixteen fat samples were obtained.

The sixteen fats that were obtained were then injected into the FTIR device. The data used was the average of values obtained. The data obtained from FTIR was further processed using IR software. The graphic display of the sixteen fats is presented in Fig. 1.

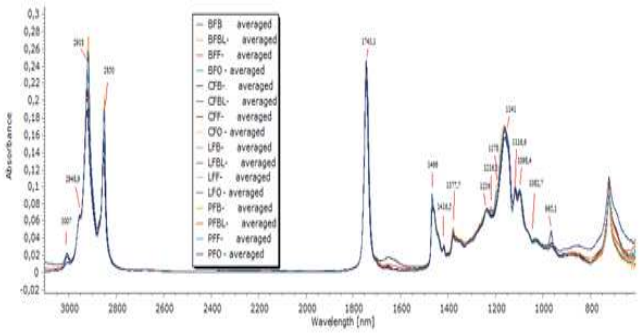

Fig. 1 FTIR spectra of lipid fraction extracted from sixteen samples averaged in infrared region (4,000 – 650 cm-1).

Fig. 1 above shows the sixteen fat IR readings in one chart. Four animal fats were used: firstly, pig fat denoted PF processed with oven (PFO), baked (PFB), fried (PFF), and boiled (PFBL), secondly, chicken fat denoted CF processed by oven (CFO), baked (CFB), fried (CFF), and boiled (CFBL), thirdly, beef fat abbreviated to BF processed with oven (BFO), baked (BFB), fried (BFF), and boiled (BFBL) and fourthly, lamb fat shortened to LF processed by oven (LFO), baked (LFB), fried (LFF), and boiled (LFBL).

TABLE 1
SPECTRUM RANGE OF FTIR IN RELATION TO FUNCTIONAL GROUPS

No	Frequency range	Description
1	***3006 – 3000 cm^{-1}***	Frequency at 3007 cm^{-1} was attributed to –C=CH (*cis* double bond stretching) and can be attributed to mono-unsaturated fatty acids (MUFA).
2	***1650 – 1645 cm^{-1}***	The C=O group of triglycerides shows a stretching vibration band at approximately 1744 cm^{-1}. The C=C stretching mode of unconjugated olefins usually shows moderate to weak absorption at 1667 – 1640 cm^{-1}. Unsubstituted trans-olefin absorbs 1670 cm^{-1}, but the band may be extremely weak or absent; unsubstituted cis-olefins absorb near 1650 cm^{-1}, and the absorption of this band is stronger than that of trans-olefin. For these reasons, these bands can be attributed to C=C stretching vibration of disubstituted cis C=C of acyl group of oleic acid and linoleic acid.
3	***1380 – 1360 cm^{-1}***	The bands between 1400 – 1000 cm^{-1} were the most difficult to assign; at approximately 1464 cm^{-1}, all spectra showed the scissoring band of the bending vibration of the methylene group. In all samples near 1400 cm^{-1} and at 1377 cm-1, a small band was observed, which was difficult to assign. This could be due to symmetrical bonding vibration of methyl group
4	***1230 – 1228 cm^{-1}***	In this region, we can see slight changes in the height of the peaks at 1200 – 1250 cm^{-1} frequency region. In general, twisting and wagging vibration of the CH_2 groups was observed in the zone between 1250 – 1150 cm^{-1} and these bands generally result from methylene scissoring.
5	***1119 – 1096 cm^{-1}***	In this frequency, pure lard showed two overlapping peaks having maxima at 1098.69 cm^{-1} and 1116.88 cm^{-1}. These peaks have been found to be inversely related to the proportion of saturated acyl group and oleic acyl groups, respectively.

The sixteen wavelengths along the spectrum were analysed using the spectra analysis software to determine the value of each of the specified target wavelengths. Location of the target wavelength and its values are listed in Table IIA and IIB.

TABLE IIA
THE SIXTEEN WAVELENGHTS FTIR VALUE OF SIXTEEN FAT SAMPLES OF FOUR PROCESSES INFRARED REGION (4,000 – 1400 CM-1).

Groups	3007	2948,9	2918	2850	1743,1	1466	1416,5	1377,7
BF-B	0,01147	0,06338	0,2572	0,1926	0,2357	0,09129	0,03138	0,04928
BF-BL	0,01083	0,06199	0,2734	0,2046	0,2337	0,09152	0,03161	0,05261
BF-F	0,01257	0,06242	0,251	0,188	0,2288	0,09017	0,03187	0,0495
BF-O	0,01158	0,06344	0,259	0,1943	0,2398	0,09158	0,03097	0,04852
CF-B	0,01929	0,06715	0,1964	0,1347	0,243	0,07331	0,0299	0,04349
CF-BL	0,01939	0,06712	0,194	0,1367	0,2419	0,07407	0,02897	0,04418
CF-F	0,01943	0,06666	0,1936	0,1367	0,2438	0,07378	0,02893	0,04374
CF-O	0,0192	0,0671	0,1968	0,1389	0,2462	0,07445	0,02904	0,04473
LF-B	0,01253	0,06416	0,2241	0,1618	0,2419	0,0802	0,02927	0,04584
LF-BL	0,01242	0,06372	0,2198	0,1586	0,2408	0,079	0,02874	0,04466
LF-F	0,01323	0,06532	0,2108	0,1493	0,2436	0,07608	0,02841	0,0444
LF-O	0,01343	0,06337	0,2129	0,1586	0,2406	0,07991	0,02827	0,04411
PF-B	0,01827	0,06584	0,2002	0,1416	0,2467	0,07438	0,02858	0,04381
PF-BL	0,01854	0,06569	0,1989	0,1406	0,2451	0,07414	0,02857	0,04378
PF-F	0,01873	0,06601	0,1988	0,1405	0,2455	0,07416	0,02861	0,04383
PF-O	0,0189	0,06637	0,1993	0,141	0,2461	0,07463	0,02887	0,04416

TABLE IIB
THE SIXTEEN WAVELENGHTS FTIR VALUE OF SIXTEEN FAT SAMPLES OF FOUR PROCESSES INFRARED REGION (1400 – 650 CM-1).

Groups	1236	1216,3	1178	1141	1116,6	1098,4	1082,7	965,1
BF-B	0,07368	0,07291	0,1382	0,1274	0,09681	0,09439	0,06057	0,03434
BF-BL	0,07511	0,07645	0,1514	0,1258	0,09573	0,09398	0,05926	0,03368
BF-F	0,07318	0,07263	0,1368	0,1254	0,09565	0,09351	0,06056	0,03516
BF-O	0,07361	0,07199	0,1374	0,1282	0,09703	0,09407	0,06127	0,03136
CF-B	0,073	0,06546	0,1224	0,1335	0,09528	0,09466	0,07185	0,03043
CF-BL	0,07313	0,06545	0,121	0,1399	0,09675	0,09364	0,07098	0,0308
CF-F	0,07332	0,06528	0,1216	0,1412	0,09747	0,09419	0,07159	0,03066
CF-O	0,07394	0,06657	0,1221	0,1414	0,09809	0,09467	0,07142	0,0306
LF-B	0,07264	0,06873	0,1268	0,1357	0,09951	0,09489	0,06471	0,04556
LF-BL	0,07183	0,06724	0,1242	0,136	0,09951	0,09443	0,0646	0,04564
LF-F	0,07191	0,06661	0,1198	0,1383	0,1003	0,09452	0,06649	0,04867
LF-O	0,07117	0,06658	0,1262	0,1383	0,09611	0,09593	0,0659	0,04871
PF-B	0,0727	0,06623	0,1202	0,1404	0,09801	0,09449	0,06918	0,02984
PF-BL	0,07275	0,06623	0,1203	0,1399	0,09763	0,09431	0,06927	0,03009
PF-F	0,07283	0,06623	0,1205	0,1401	0,09762	0,09445	0,06954	0,03022
PF-O	0,07306	0,06632	0,1209	0,1404	0,09793	0,09466	0,07023	0,03025

Table IIA and IIB show the 256 values corresponding to four types of animal fats processed via four different processes. It was expected that these processes do not change the chemical structure of the animal fats. Minitab 17 was used to analyse the data. The results of the analysis can be seen in Fig. 2.

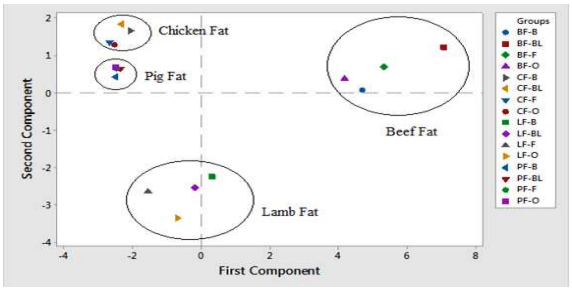

Fig. 2 Score plot of sixteen fat animal groups in sixteen wavelengths in the specified fat chart

Fig. 2 shows the score plot for the animal fats processed differently (via oven, baked, fried, and boiled) remains grouped within the same type of animal fat. This suggest that processing did not cause structural changes in the fat derived from the four types of animal meat.

Following this, only data from the oven process across the four types of meat was used in the next biomarker identification step: pig fat processed via oven (PF-O), chicken fat processed via oven (CF-O), beef fat processed via oven (BF -O), and lamb fat processed via oven (LF-O). Palm oil (PO), was added to the analysis; 5 samples were used in total.

B. Pig Biomarker Identification

Identification of pig biomarker was carried out on the four animal fats obtained via oven process and palm oil. Each sample was analysed five times; biomarker value was recorded as the average of the 5 repetition values.

Fig. 3 shows the overall spectrum of the fats tested (4000-650 nm). The region of interest in the functional group region included wavelengths from 3000 to 2800 nm, while in the fingerprint region included 1800-900 nm.

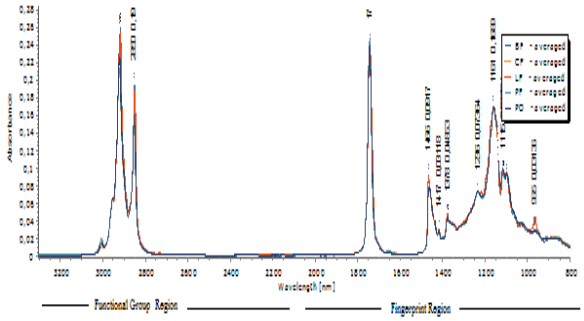

Fig. 3 FTIR spectra of lipid fraction extracted of 5 biomarker samples averaged infrared region (4,000 – 650 cm-1).

Five fat samples and sixteen wavelenghts were discerned for use as biomarkers. The sixteen wavelenghts identified included four wavelenghts in the functional group region and twelve wavelenghts in the fingerprint region. The values were determined directly using software. The values of the sixteen wavelenghts are summarized in Table IIIA and IIIB.

TABLE IIIA
THE SIXTEEN WAVELENGHTS FTIR VALUE OF FIVE FAT SAMPLES OF OVEN PROCESS INFRARED REGION (4,000 –1400 CM-1).

	Functional Groups				Finger Print			
	3007	2948.9	2918	2850	1743.1	1466	1416.5	1377.7
BF	0.01158	0.06344	0.259	0.1943	0.2398	0.09155	0.03095	0.04852
CF	0.0192	0.06706	0.1967	0.1392	0.2462	0.07448	0.02901	0.04472
LF	0.01173	0.06336	0.2529	0.1887	0.2407	0.08989	0.03124	0.04811
PF	0.01891	0.06633	0.1992	0.1413	0.2461	0.07467	0.02884	0.04415
PO	0.01521	0.06598	0.211	0.1505	0.2414	0.07704	0.02896	0.04531

TABLE IIIB
THE SIXTEEN WAVELENGHTS FTIR VALUE OF FIVE FAT SAMPLES OF OVEN PROCESS INFRARED REGION (1400 – 650 CM-1).

	Finger Print							
	1236	1216.3	1178	1141	1116.6	1098.4	1082.7	965.1
BF	0.07361	0.07199	0.1374	0.128	0.097	0.09407	0.06134	0.03136
CF	0.07394	0.06657	0.122	0.1412	0.0981	0.09469	0.0715	0.0306
LF	0.07417	0.07258	0.1361	0.128	0.09608	0.09593	0.06097	0.04871
PF	0.07307	0.06632	0.1208	0.1402	0.09793	0.09469	0.07031	0.03025
PO	0.07387	0.06715	0.1229	0.1373	0.1009	0.09335	0.06858	0.02939

Prominent peaks in the functional group region were at wavelenghts 3007 nm, 2948.9 nm, 2918 nm and 2850 nm. Fig. 4a shows four spectra of the five sample values in the functional group region (4000 – 2000 nm). The first spectrum shows wavelength 3007 of the five values, the second spectrum shows wavelength 2948.9, the third spectrum shows wavelength 2918, and the fourth spectrum shows wavelength 2850.

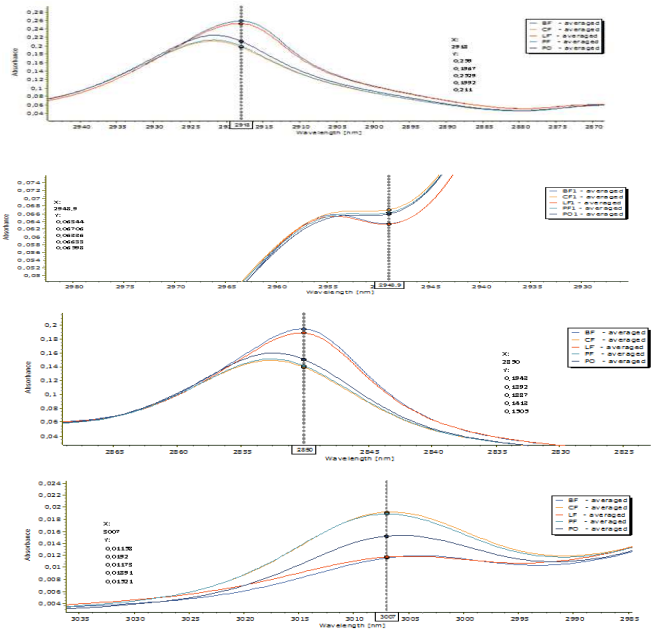

Fig. 4a FTIR spectra of lipid fraction extracted of 5 biomarker samples averaged and its value infrared functional group region (4,000 – 2000 nm).

Fig. 4b, 4c and 4d show spectra of the five sample values in the fingerprint group region (2000 – 650 nm). Twelve prominent wavelengths were identified: wavelengths 1743.1, 1466, 1416.5, 1377.7, 1236, 1216.3, 1178, 1141, 1116.6, 1098.4, 1082.7 and 965.1. The spectrum of the five samples at each wavelength in the fingerprint region is shown in Fig. 4b, 4c and 4d.

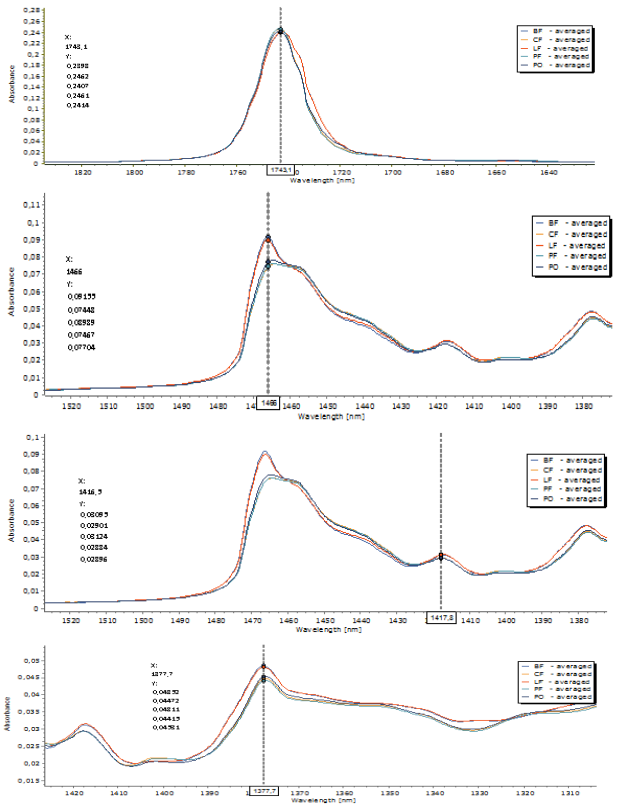

Fig. 4b FTIR spectra of lipid fraction extractedof 5 biomarker samples averaged and its value infrared fingerprint group region (2000 - 650 nm)

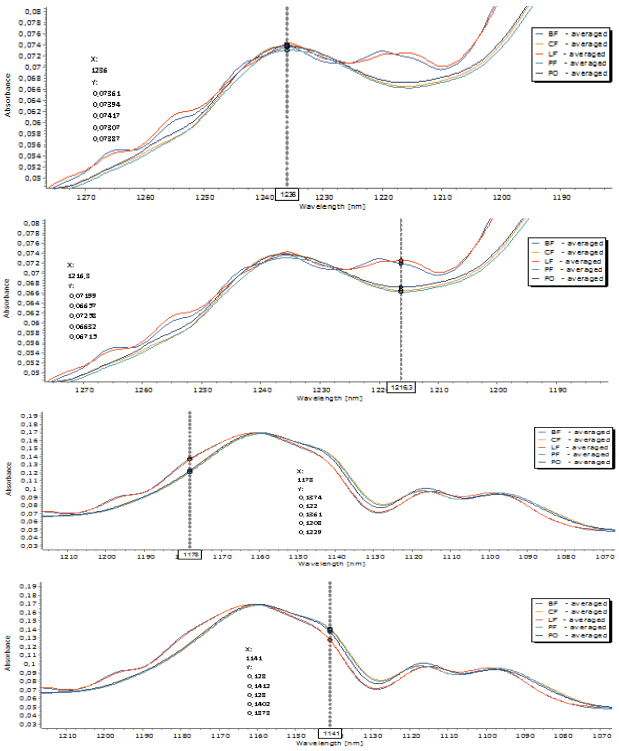

Fig. 4c FTIR spectra of lipid fraction extracted of 5 biomarker samples averaged and its value infrared fingerprint group region (2000 - 650 nm).

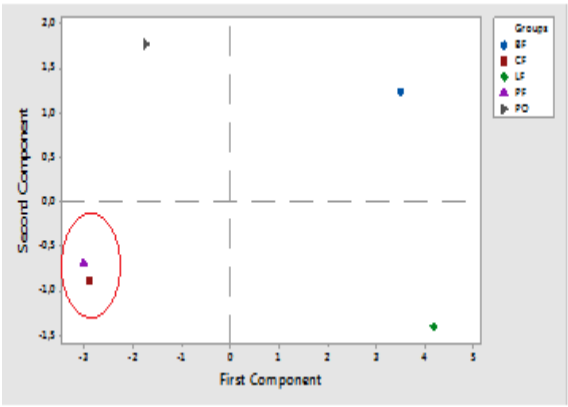

Fig. 5 Score plot of five fat animal groups in sixteen wavelengths in the specified fat chart

Figure 5 shows that the sixteen wavelengths in the spectrum can be plotted to distinguish pig fat against beef fat, lamb fat, and palm oil, but not pig fat against chicken fat. The biomarkers for pig and chicken fats were visually similar, therefore it was predicted that it would be difficult to identify samples containing pork fat from chicken fat from these wavelengths. However, this problem was overcome by using a scatterplot screener program. The program compared between two wavelengths across the sixteen wavelengths, and significant wavelengths differentiating pig fat from chicken fat were identified. The sixteen scatterplot screener values were calculated to determine wavelengths in the spectrum that would be able to properly distinguish pig fat from chicken fat. The results of the scatterplot screener calculations can be seen in Table IVa and IVb.

Table IVA and IVB shows comparison between two spectrum wavelengths denoted with colour codes. The green boxes indicate excellent wavelengths for use as biomarker, the yellow boxes indicate average wavelengths for use as biomarker, and the orange and red boxes indicate poor wavelengths for use as biomarker.

Wavelengths 3007 to 965.1 paired against wavelength 1236 resulted in green boxes in the table, meaning all the fats and oil separated well. The wavelength which resulted in the best separation against wavelength 1236 is wavelength 3007. These two wavelengths not only significantly differentiated pig fat from chicken fat, but were excellent at separating the two animal fats from palm oil as well.

Differences in wavelengths green, yellow, orange, and red can be clearly seen when scatter plots are applied. The five fats and oil that were identified to have good distance are circled in red. Examples of wavelength pairings resulting in red wavelengths, yellow wavelengths and green wavelengths are shown in Fig. 6, 7, 8 and 9 respectively.

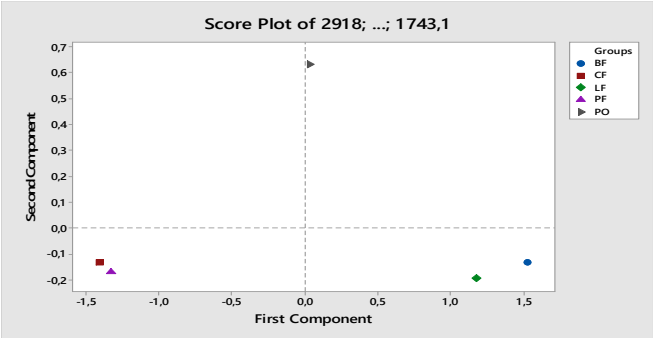

Fig. 6 Score plot of four fat animal and one palm oil in sixteen wavelengths (from 2918 to1743,1)nm in the specified fat chart

Fig. 7 shows the results of plot scores of the four animal fats and palm oil at wavelengths 2918 and 1743.1 nm on the spectrum (red box in Table 3); the biomarker wavelengths differentiating pig fat from chicken fat were relatively close compared to the wavelengths between pig fat with beef fat, lamb fat, and palm oil.

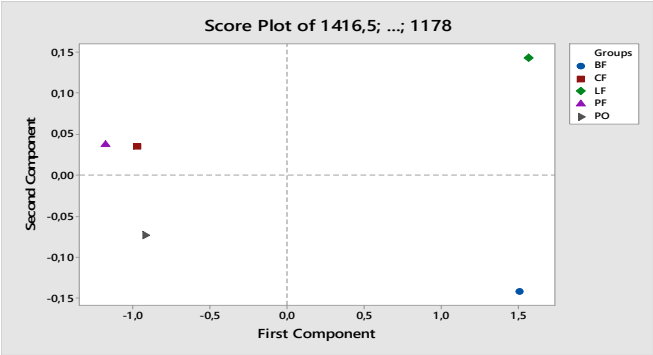

Fig. 7 Score plot of four fat animal and one palm oil in two wavelengths (1416,5 and 1178)nm in the specified fat chart

Fig. 8 shows the results of plot scores of the four animal fats and palm oil wavelengths 1416.5 and 1178 nm on the spectrum (red box in Table III); visually, the pig and chicken fat biomarker wavelengths were still relatively close compared to the wavelengths between pig fat and beef fat, lamb fat, and palm oil that are relatively farther.

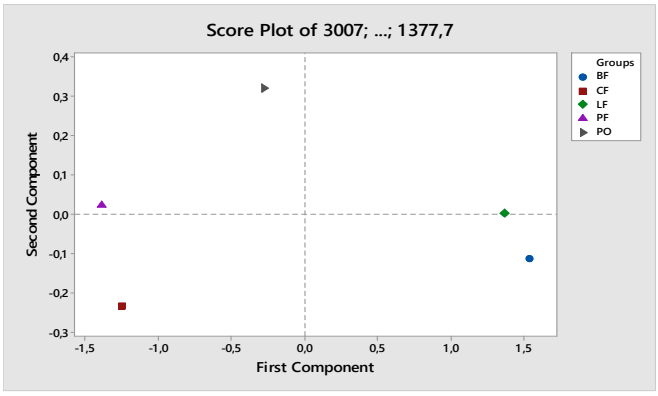

Fig. 8 Score plot of four fat animal and one palm oil in two wavelengths (1377,7 and 3007)nm in the specified fat chart

Fig. 9 shows the results of plot scores of the four animal fats and palm oil wavelengths 3007 and 1377.7 nm on the spectrum (yellow box in Table IV); it is observed that the pig and chicken biomarker wavelengths are farther apart; similarly, biomarker wavelengths identifying pig fat against beef fat, lamb fat, and palm oil are located far apart. Therefore, when using these two wavelengths for fat identification, the five fats and oil could be well separated.

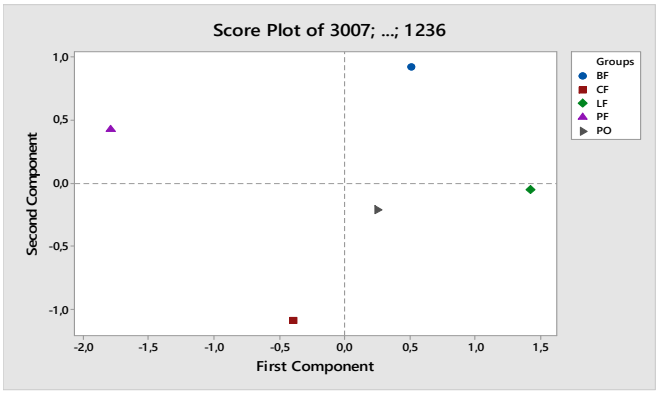

Fig. 9 Score plot of four fat animal and one palm oil in two wavelengths (1236 and 3007)nm in the specified fat chart

Fig. 10 shows the results of plot scores of the four animal fats and palm oil at wavelengths 1236 and 3007 nm on the spectrum (green box in Table IV); it is observed that the pig and chicken biomarker wavelengths, as well as that of pigs biomarker wavelengths against beef fat, lamb fat, and palm oil are well- separated in distance from one another. Therefore, using these two wavelengths for identification of the five fats and oil would result in clear separation.

Among the fifteen green wavelengths present in Table IV, wavelengths 1236 and 3007 were the most appropriate as biomarker wavelengths, because the pig fat and chicken fat wavelengths were located significantly far enough to be distinguished, as well as between pig fat against beef fat, lamb fat, and palm oil. The differences in the paired wavelengths can be seen in Fig. 10.

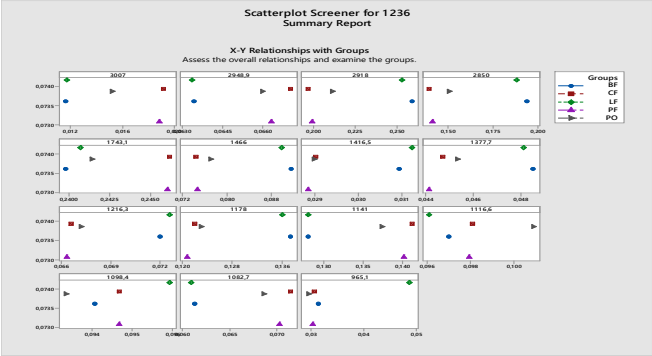

Fig. 10 Scatterplot screener of four fat animal and one palm oil in two wavelengths (1236 and all) nm in the specified fat chart

The two prominent frequencies were at wavelengths 3007 and 1236; frequency at 3007 cm^{-1} was attributed to –C=CH (*cis* double bond stretching) and can be correlated to mono-unsaturated fatty acids (MUFA). Meanwhile, at frequency range 1230 – 1228 cm^{-1}, slight changes in the height of the peaks at the 1200 – 1250 cm^{-1} region was observed. In general, twisting and wagging vibration of the CH_2 groups was observed in the zone between 1250 – 1150 cm^{-1}; these bands are generally the result of methylene scissoring.

IV. CONCLUSIONS

The biomarker wavelengths identified from the spectra of the four fats and palm oil at position 1236 and 3007 cm-1 separated the four animal fats and palm oil at notable distances, indicating that these wavelength could be used to identify non-halal samples.

TABLE IVA
SPECTRUM WAVELENGTH CALCULATION

	3007	2948.9	2918	2850	1743.1	1466	1416.5	1377.7
3007								
2948.9								
2918								
2850								
1743.1								
1466								
1416.5								
1377.7								
1236								
1216.3								
1178								
1141								
1116.6								
1098.4								
1082.7								
965.1								

TABLE IVB
SPECTRUM WAVELENGTH CALCULATION

	1236	1216.3	1178	1141	1116.6	1098.4	1082..7	965.1
3007								
2948.9								
2918								
2850								
1743.1								
1466								
1416.5								
1377.7								
1236								
1216.3								
1178								
1141								
1116.6								
1098.4								
1082.7								
965.1								

ACKNOWLEDGMENTS

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