

Identification of Pig Adulterant in Mixture of Fat Samples and Selected Foods based on FTIR-PCA Wavelength Biomarker Profile

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Abstract— Authenticity is an important issue in food industry. Tampering the authenticity of food product involves the adulteration of products with certain material. Various authentication techniques for detection of adulteration have been developed in line with the advent of current technology. Of particular interest, Infrared (IR) spectroscopy; a rapid and non-destructive technique allowing the screening of a large number of samples has been shown to be able to detect pig derivatives in meat products. Following this, the present study aims to identify pig adulteration in different mixture of fat samples and some selected food; based on wavelength biomarker obtained from FTIR coupled with PCA analysis. Twenty-six fats at two frequencies along the graph (1236 and 3007 nm) were studied including samples representing Non Halal Food A (NHFA) fat, Halal Food A(HFA) fat and Non Halal Food B (NHFB) fat. At wavelength 1236 and 3007 nm along the spectrum; NHFA, HA and NHFB fat samples were easily identified at visibly good distance compared to other fat samples. The first two samples; NHFA and NHFB were located very close to PF (Pig Fat) indicating that NHFA and NHFB samples contained pork fat while HA was located closer to CF, indicating that the sample possibly contained chicken fat. To this end, FTIR coupled with PCA has been shown to be a powerful tool to detect adulteration in meat products and as such can be recommended for authentication purposes.

Keywords— Pig; biomarker; FTIR; PCA; authentication; fat; halal food.

I. INTRODUCTION

The production of food has evolved in line with modern advancements in science and technology. Various ingredient sources are being used in the production of food. These ingredients may be either permissible (*halal*) or prohibited (*haram*) [5].

Adulteration is defined as the addition of undeclared substances or materials to a product so as to increase bulk product or weight, making the product appear more valuable than it actually is [6]. In the case of meat and meat articles, adulteration not only refers to the replacement of ingredients but also to inappropriate information concerning the origin of raw materials [8]. Some halal meat issues that have arisen are the mixing of meats from halal and haram sources involving two types of animals: expensive and halal meat mixed with cheap and haram meat. For example, the mixing of beef and pork meats is often done by butchers solely for the benefit of gaining extra profit because pork is cheaper than

beef. Visual inspection alone is impossible to differentiate between beef and pork meats.

The development of current technology enables the food product to be accurately analysed in terms of its contents and therefore the determination of illegal adulterants in halal products can be done effectively [7]. Scientists have introduced various halal authentication techniques. Enzyme Linked Immunosorbent Assays (ELISA), Radio Immunoassays (RIA), HPLC, FTIR, Electronic Nose coupled with GC-MS and PCR assays have been applied to identify biomarkers, pathogens or chemicals in processed and unprocessed food including meat; that help in determining the halal status. The use of instruments such as Fourier Transform Infrared (FTIR) spectroscopy to detect pig derivatives in meat products is previously described [2].

FTIR is a technique that measures the vibration of the bonds in molecular functional groups [3]. Infrared (IR) light is used to generate information on the molecular composition and structure of various types of materials including fats and oils. Combination of FTIR techniques and

chemometric analysis have been reported to be to detect and measure pig fat levels in food samples [10].

Chemometrics is the chemical discipline that uses mathematics and statistics to design or select optimal experimental procedures, provide maximum relevant chemical information by analyzing chemical data and obtain knowledge about chemical systems. Principal component analysis (PCA) is often used in chemometric analysis. It is a method of data processing whereby a small number of synthetic variables called principal components are extracted from a large number of variables measured in order to explain a certain phenomenon [4],[12].

This study aims to identify pig adulteration in different mixture of fat samples and some selected food; based on wavelength biomarker obtained from FTIR coupled with PCA analysis.

II. MATERIAL AND METHOD

A. Sample Preparation and Extraction

1) *Preparation and Extraction of Fat Samples:* A total of four meat samples from pig, chicken, lamb and beef were collected from Gombak market in Selangor, Malaysia. The preparation started firstly by washing the samples using distilled water to remove any contamination on the surface of the meat samples. Then, the meat samples were cut into small sizes (1 cm x 1 cm) and kept at -20 °C until use.

The fat samples of the meat (pig, chicken, beef, lamb) was prepared by rendering adipose tissue of animal according to previously reported procedure by Rohman and Che Man [9]. In this process, the meat was cut into small pieces, mixed, and melted at 90–100° C for 2 h in the oven. The melted fat was strained through triple-folded muslin cloth, dried by addition of anhydrous Na₂SO₄ and then centrifuged at 3000 rpm for 20 min. The fat layer was decanted, shaken well and centrifuged again before being filtered through Whatman filter paper containing sodium sulfate anhydrous to remove trace of water. The prepared oils were then used for FTIR and GC analyses or kept in tightly closed containers under a nitrogen blanket at -20 °C [3].

2) *Calibration and Validation:* For calibration model, a set of standards consisting of pig fat in palm oil and pig fat in chicken fat was made by blending both fats at concentration ranges of 10, 20, 30, 40, 50, 60, 70, 80 and 90% (v/v) of pig fat in the other fat/oil. For validation/prediction, a series of independent samples, which were different from calibration samples were constructed. Pig fat and palm oil as well as their blends in neat form were analyzed using FTIR spectrophotometer. The spectral regions where variations among the fats (were observed) were chosen for developing multivariate analysis.

3) *Food Samples Preparation and Extraction:* A total of three food samples (Non Halal Food A; NHFA, Halal Food A; HFA, and Non Halal Food B; NHFB) containing certain animal meats were collected from a local market at Gombak in Malaysia. One sample was prepared from each food type. The preparation started firstly by washing the samples with distilled water to remove any contamination present on the surface of the meat samples. Then, the meat samples were cut into small sizes (1 cm x 1 cm) and kept at -20 °C until they were used for the fat extraction process.

Fat in the food samples were extracted by rendering the samples according to the method described by Rohman and Che Man [9]. All chemicals used in this experiment were of analytical grade. The pure extracted fats were then analyzed by means of FTIR spectroscopy.

B. Analysis Using FTIR Spectroscopy

Nicolet iS50 FTIR Spectrometer was used to acquire the full spectrum in the mid infrared region (400-4000 cm⁻¹). The number of scans was fixed to 32 with a resolution of 4 cm⁻¹. The measurement was calibrated against a blank background. The whole FTIR spectrum corresponded to the stretching of the functional groups present in the fat samples. The graph shows the average spectrum of four samples: pig fat, chicken fat, beef fat, lamb fat, and palm oil. The fats from food samples were analysed as well. Each sample was analyzed five times using FTIR.

C. Spectral Analysis.

The raw FTIR spectra were smoothed and their baseline corrected and normalized using the freeware software *SpectraGryph 1.2.8*.

D. Statistical Analysis.

Principal Component Analysis (PCA) was carried out based on [11]. Scatter plot screener program and table analysis were also used.

III. RESULTS AND DISCUSSION

A. Calibration Model; a Set of Standards Consisting of 'Pig Fat in Chicken Fat' (PC)

PC1, PC2, PC3, PC4, PC5, PC6, PC7, PC8, PC9 (10, 20, 30, 40, 50, 60, 70, 80, 90)% and BF (beef fat), CF (chicken fat), LF (lamb fat), PF (pig fat) and PO (palm oil) (100)% were prepared and injected into the FTIR device. Each fat was injected five times. Values reported were the average of the 5 replicates. Data obtained from FTIR was further processed using infrared reader software. The spectrum display of the fourteen fats can be seen in the following Fig. 1.

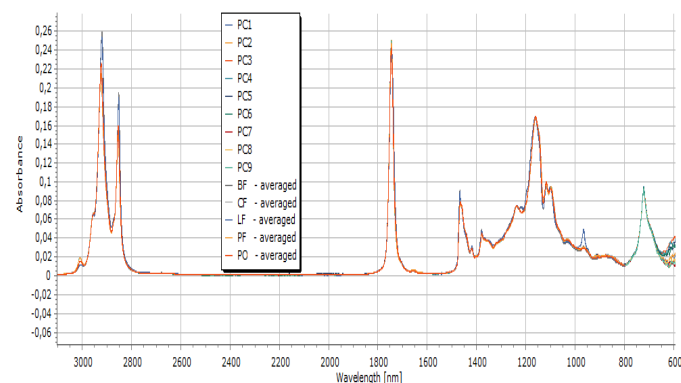


Fig.1 FTIR spectra of lipid fraction extracted from sixteen samples averaged of PF (pig fat) and CF (chicken fat) mixture in infrared region (4,000 – 650 cm⁻¹).

Sixteen wavelengths of interest were identified: four wavelengths in the functional group region and twelve wavelengths in the fingerprint region. Fig.1 shows the position of each wavelength relative to each other. The values were determined using software. The values of the sixteen wavelengths are summarized in the following Table IA and IB.

TABLE I A
THE SIXTEEN FTIR WAVELENGTH VALUES OF FOURTEEN PF (PIG FAT) AND CF (CHICKEN FAT) BLENDS LOCATED IN THE 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
PC1	0.01921	0.06721	0.1976	0.1395	0.2491	0.07432	0.02878	0.04451
PC2	0.01915	0.06727	0.198	0.1399	0.2494	0.07462	0.02901	0.04462
PC3	0.01912	0.06709	0.1981	0.14	0.2492	0.07481	0.0292	0.04483
PC4	0.01904	0.06701	0.1983	0.1401	0.2493	0.07477	0.02911	0.04465
PC5	0.01915	0.06706	0.1987	0.1405	0.2493	0.07489	0.02915	0.04471
PC6	0.01896	0.06676	0.199	0.1407	0.2489	0.07481	0.02906	0.0445
PC7	0.01916	0.06697	0.1993	0.141	0.2489	0.0747	0.02896	0.04431
PC8	0.01921	0.06736	0.1998	0.1412	0.2468	0.07477	0.0289	0.04449
PC9	0.01914	0.06745	0.2004	0.1416	0.2487	0.07487	0.02878	0.04431

TABLE I B
THE SIXTEEN FTIR WAVELENGTH VALUES OF FOURTEEN PF (PIG FAT) AND CF (CHICKEN FAT) BLENDS LOCATED IN THE 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
PC1	0.07378	0.06648	0.1219	0.1419	0.09822	0.09469	0.07132	0.03014
PC2	0.07389	0.06652	0.122	0.1421	0.0982	0.09489	0.07116	0.03044
PC3	0.07391	0.06678	0.1221	0.1422	0.09865	0.09533	0.07156	0.03085
PC4	0.07379	0.06662	0.1221	0.142	0.09859	0.09518	0.07125	0.03053
PC5	0.07373	0.06668	0.1219	0.1419	0.09856	0.09516	0.07111	0.03066
PC6	0.07348	0.06654	0.1217	0.1416	0.09831	0.09505	0.07064	0.03049
PC7	0.07337	0.06635	0.1214	0.1412	0.09821	0.09482	0.0704	0.03018
PC8	0.0733	0.06637	0.1215	0.1405	0.09784	0.09487	0.07039	0.03012
PC9	0.07317	0.06627	0.1212	0.1409	0.09797	0.09482	0.07014	0.0301

All the values in Table IA and IB were entered in the reader software to display the scatter plot image of the wavelengths as a whole. The result can be seen in the following score plot in Fig.2.

Fig. 2 shows that the sixteen wavelengths in the spectrum were able to separate pig fat (PF) from beef fat (BF), lamb fat (LF), and palm oil (PO) but not pig fat (PF) from chicken fat (CF). Visual inspection showed that wavelengths of pig fat and chicken fat were very close rendering it difficult to

use these wavelengths to identify samples containing pork and chicken.

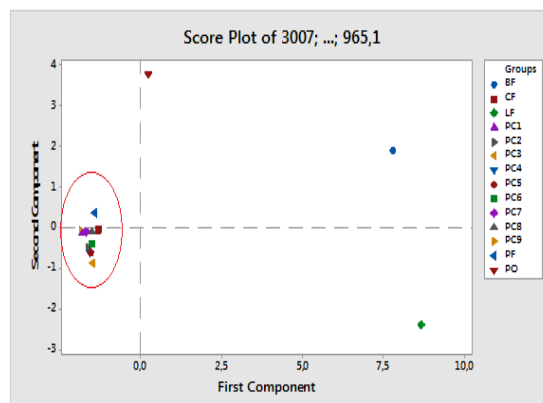


Fig. 2 Score plot of sixteen wavelength of fourteen samples of PF (pig fat) and CF (chicken fat) mixture

Fig. 2 above shows that the sixteen wavelengths in the spectrum were able to separate pig fat (PF) from beef fat (BF), lamb fat (LF), and palm oil (PO) but not pig fat (PF) from chicken fat (CF). Visual inspection showed that wavelengths of pig fat and chicken fat were very close rendering it difficult to use these wavelengths to identify samples containing pork and chicken.

At frequency 1236 and 3007 nm of the score plots, the biomarker wavelengths for pig and chicken fat as well as pig fat and beef fat, lamb fat and palm oil were located distinctly far away. Using these two wavelengths for identification of all the fats in food samples would sufficiently distinguish between the fats and oil.

The values at frequency 1236 and 3007 nm in Table II were entered in the reader software to display the whole scatter plot image. The resulting analysis can be seen in the following score plot Fig. 3.

TABLE II
VALUE WAVELENGTHS FOR FTIR PEAKS AT 1236 AND 3007 NM. VALUES ARE OF FOURTEEN MIXTURE FAT SAMPLES OF PF AND CF IN THE INFRARED REGION (4,000 – 650 cm^{-1}).

Groups	3007	1236
BF	0.01158	0.07361
CF	0.0192	0.07394
LF	0.01173	0.07417
PF	0.01891	0.07307
PO	0.01521	0.07387
PC1	0.01921	0.07378
PC2	0.01915	0.07389
PC3	0.01912	0.07391
PC4	0.01904	0.07379
PC5	0.01915	0.07373
PC6	0.01896	0.07348
PC7	0.01916	0.07337
PC8	0.01921	0.0733
PC9	0.01914	0.07317

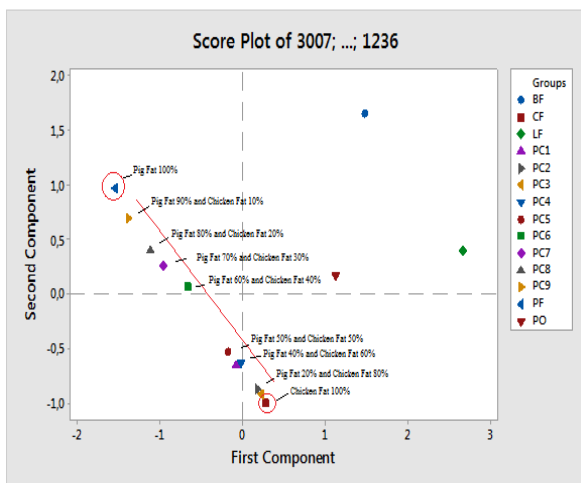


Fig. 3 Score plot of two wavelength of fourteen samples of PF (pig fat) and CF (chicken fat) mixture

Fig. 3 shows the wavelength values at frequency 3007 and 1236 nm for pig fat and chicken fat samples at mixed concentrations. The wavelengths formed a linear line, unlike in Fig. 3 prior to using the scatter plot program where the wavelengths are stacked. The linear line would facilitate the identification and calculation of the concentration of food samples in future analyses.

B. Calibration Mode; a Set of Standards Consisting of Pig Fat in Palm Oil

The four fats, palm oil and nine pig fat in palm oil blends samples (10, 20, 30, 40, 50, 60, 70, 80, 90)% were prepared and injected into the FTIR device. Each fat was injected five times. The values reported were average values of the five replicates. Data obtained from FTIR was further processed using infrared reader software. The spectrum display of the fourteen fats are shown in the following Fig.4.

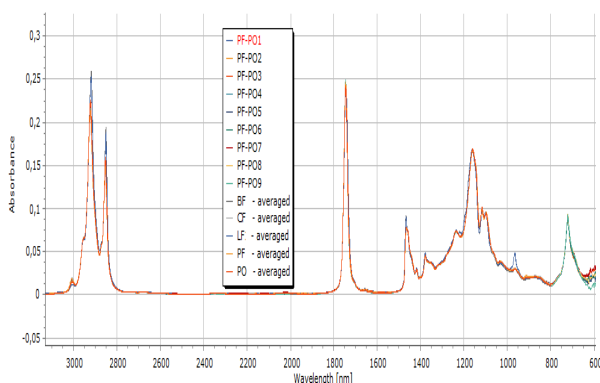


Fig. 4 FTIR spectra of lipid fraction extracted from sixteen samples averaged of PF (pig fat) and PO (palm oil) mixture in infrared region (4.000 – 650 cm^{-1}).

Sixteen wavelengths were identified which include four wavelengths in the functional group region and twelve wavelengths in the fingerprint region. Fig. 4 shows the peak positions of each fat sample relative to each other for comparison purposes. These values were determined directly using the software. The values of the sixteen wavelengths are summarized in the following Table IIIA and IIIB.

TABLE III A
THE SIXTEEN WAVELENGTH FTIR VALUE OF FOURTEEN MIXURE FAT SAMPLES OF PF AND PO 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
PF-PO1	0.01578	0.06551	0.2084	0.1481	0.2373	0.07656	0.02894	0.04525
PF-PO2	0.01601	0.06671	0.2091	0.1483	0.2388	0.07672	0.02894	0.0452
PF-PO3	0.01604	0.0657	0.2083	0.148	0.2388	0.0767	0.02913	0.0453
PF-PO4	0.0164	0.06602	0.2071	0.147	0.2413	0.07624	0.02884	0.04495
PF-PO5	0.01704	0.06633	0.2061	0.1461	0.2454	0.07599	0.02891	0.04478
PF-PO6	0.01715	0.06609	0.2053	0.1456	0.2457	0.07588	0.02886	0.0447
PF-PO7	0.01799	0.06646	0.2037	0.1443	0.2473	0.07579	0.02922	0.04477
PF-PO8	0.01832	0.06637	0.2021	0.1431	0.248	0.07526	0.0289	0.0444
PF-PO9	0.01866	0.06656	0.2012	0.1423	0.2484	0.07487	0.0287	0.04417

TABLE III B
THE SIXTEEN WAVELENGTH FTIR VALUE OF FOURTEEN MIXURE FAT SAMPLES OF PF AND PO 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
PF-PO1	0.07373	0.06702	0.1226	0.1364	0.1002	0.09291	0.06851	0.02958
PF-PO2	0.07372	0.06705	0.1227	0.1369	0.1001	0.0932	0.06869	0.0297
PF-PO3	0.0738	0.06709	0.1228	0.1369	0.1002	0.09342	0.06892	0.0297
PF-PO4	0.07361	0.06684	0.1225	0.1378	0.09968	0.09353	0.06882	0.02957
PF-PO5	0.07351	0.06672	0.1222	0.1393	0.09969	0.09416	0.06925	0.02965
PF-PO6	0.07341	0.06678	0.1219	0.1395	0.09944	0.09417	0.06951	0.0297
PF-PO7	0.07352	0.06679	0.122	0.1402	0.09922	0.09474	0.07009	0.03033
PF-PO8	0.07322	0.06641	0.1215	0.1404	0.09849	0.09456	0.06991	0.03013
PF-PO9	0.07303	0.06623	0.1211	0.1407	0.09814	0.09469	0.07003	0.02966

All the values in Table III were entered in the reader software to display the scatter plot image as a whole. The resulting score plot is shown in Fig. 5.

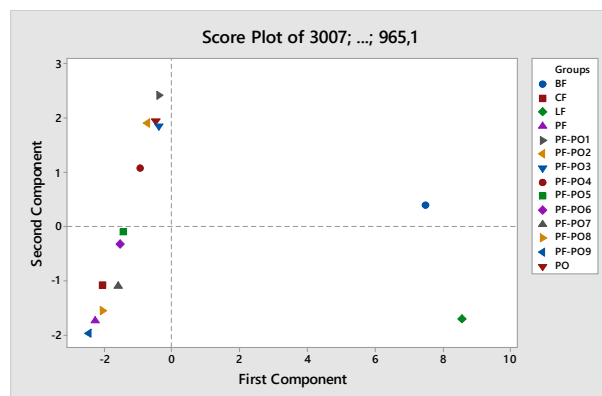


Fig. 5 Score plot of sixteen wavelength of fourteen samples of PF (pig fat) and PO (palm oil) mixture

Fig. 5 above shows that the sixteen wavelengths in the whole spectrum distinctly separated pig fat from beef fat, lamb fat and palm oil but not pig fat from chicken fat. The wavelengths for pig and chicken fats were located very close to each other. Therefore it would be difficult to identify samples containing pork and chicken using these wavelengths. This problem was solved using a scatterplot screener program. The program compared the sixteen wavelengths in pairs to identify frequency at which the biomarker wavelengths for pig fat was notably far from chicken fat.

In the plot scores of four animal fats and palm oil at two wavelengths along the graph (1236 and 3007 nm), it was seen that the pig and chicken fat biomarker wavelengths are clearly distanced. Similarly, the biomarker wavelengths between pig fat with beef fat, lamb fat and palm oil that were visually far from each other. Therefore, using these two wavelengths for identification of the five fats and palm oil would result in good separation.

TABLE IV
THE TWO WAVELENGTH FTIR VALUE OF FOURTEEN MIXTURE FAT SAMPLES OF PF AND PO INFRARED REGION (4.000 – 650 CM⁻¹).

Groups	3007	1236
BF	0.01158	0.07361
CF	0.0192	0.07394
LF	0.01173	0.07417
PF	0.01891	0.07307
PO	0.01521	0.07387
PF-PO1	0.01578	0.07373
PF-PO2	0.01601	0.07372
PF-PO3	0.01604	0.0738
PF-PO4	0.0164	0.07361
PF-PO5	0.01704	0.07351
PF-PO6	0.01715	0.07341
PF-PO7	0.01799	0.07352
PF-PO8	0.01832	0.07322
PF-PO9	0.01866	0.07303

The values for wavelengths in frequency 3007 and 1236 nm in Table IV were entered in the reader software to display the image as a whole. The resulting analysis is seen in the following score plot Fig. 6.

Values at wavelength 3007 and 1236 in Fig. 6 show the mixed concentrations of pig fat and palm oil forming a linear line. This would facilitate the identification and calculation of the concentration of target compound in food samples in future analyses.

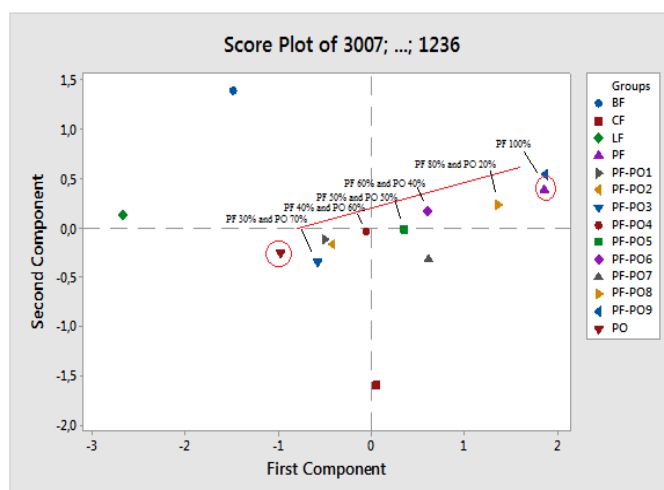


Fig. 6 Score plot of two wavelength of fourteen samples of PF and PO mixture

Values at wavelength 3007 and 1236 in Fig. 6 show the mixed concentrations of pig fat and palm oil forming a linear line. This would facilitate the identification and calculation of the concentration of target compound in food samples in future analyses.

C. Data Calibration Model; a Set of Standards Consisting of Pig Fat, Chicken Fat and Palm Oil

The calibration data of pig fat mixed with chicken fat and pig fat mixed with palm oil were combined in one picture; similarly the data in Table II and Table IV are combined into Table V. Reader software was used to display the whole image.

TABLE V
THE TWO WAVELENGTH FTIR VALUE OF TWENTYTHREE MIXTURE FAT SAMPLES OF PFCF AND PFPO INFRARED REGION (4.000 – 650 CM⁻¹).

Groups	3007	1236
BF	0.01158	0.07361
CF	0.0192	0.07394
LF	0.01173	0.07417
PF	0.01891	0.07307
PO	0.01521	0.07387
PF-PO1	0.01578	0.07373
PF-PO2	0.01601	0.07372
PF-PO3	0.01604	0.0738
PF-PO4	0.0164	0.07361
PF-PO5	0.01704	0.07351
PF-PO6	0.01715	0.07341
PF-PO7	0.01799	0.07352
PF-PO8	0.01832	0.07322
PF-PO9	0.01866	0.07303
PC1	0.01921	0.07378
PC2	0.01915	0.07389

PC3	0.01912	0.07391
PC4	0.01904	0.07379
PC5	0.01915	0.07373
PC6	0.01896	0.07348
PC7	0.01916	0.07337
PC8	0.01921	0.0733
PC9	0.01914	0.07317

Wavelength values for frequency 3007 and 1236 in Table II and Table IV were incorporated and fed into the reader software to display the whole scatter plot image. The resulting analysis is seen in the following score plot Fig. 7.

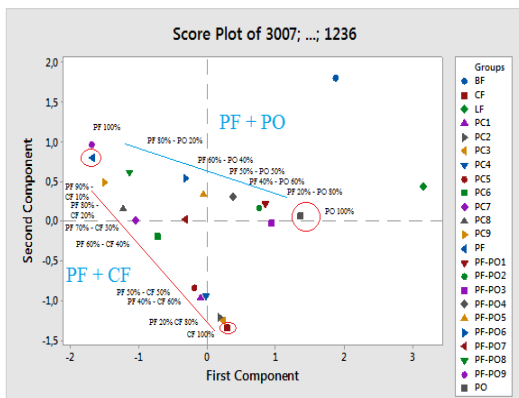


Fig. 7 Score plot at two wavelengths (3007 and 1236) of fourteen samples of PFCF mixture and PFPO mixture

The wavelength values at 3007 and 1236 nm plotted in Fig. 6 shows the concentrations of pig fat mixed with chicken fat forming a somewhat linear line. It is not possible to achieve a perfect line in mixed samples. Linear lines facilitate the identification and calculation of food samples in future analyses. Fig. 7 (in red lines) shows a mixed concentration of 90% pig fat close to 100% pig fat in decreasing concentrations until the lowest pig fat concentration close to 100% chicken fat. Fig. 7 shows mixed concentrations of pig fat and palm oil forming a somewhat linear line as above. Linear lines facilitate the identification and calculation of food samples in future analyses. Fig. 5 (in blue lines) shows a mixed concentration of 90% pig fat close to 100% pig fat and so on until the smallest pig fat concentration was close to 100% palm oil.

D. Food Sample Spectral Analysis

FTIR Spectrometer was used to acquire the full spectrum in the mid infrared region (400-4000 cm^{-1}). The whole FTIR spectrum corresponded to the stretching of the functional groups present in the fat. The graph shows the average spectrum of five spectra for NHFA, HFA and NHFB.

The three samples were injected into the FTIR device. Each fat was injected five times; the values reported were average values of the replicates. File data obtained from FTIR was further processed using infrared reader software. Graphical display of the sixteen wavelengths is shown in the following Fig. 8.

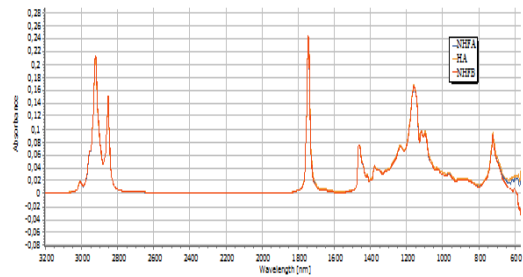


Fig. 8 FTIR spectra of lipid fraction extracted from three food fat samples NHFA (Non Halal Food A), HFA (Halal Food A) and NHFB (Non Halal Food B) in infrared region (4.000 – 650 cm^{-1}).

Sixteen wavelengths were identified which include four wavelengths in the functional group region and twelve wavelengths in the fingerprint region. The above values show the position of the five fats at different wavelengths as such that their positions can be compared against each other. These values of the sixteen wavelengths were determined directly using the software. The values can be summarized in the following Table VIA and VIB.

TABLE VIA
THE SIXTEEN WAVELENGTH FTIR VALUE OF THREE FOOD FAT SAMPLES OF NHFA.HFA. AND NHFB INFRARED REGION (4.000 – 1400 CM^{-1}).

	Functional Groups				Finger Print			
	3007	2948.9	2918	2850	1743.1	1466	1416.5	1377.7
NHFA	0.01835	0.06569	0.1995	0.1414	1.602	0.07425	0.02842	0.04374
HA	0.02001	0.06712	0.1907	0.1345	1.686	0.07378	0.03008	0.04532
NHFB	0.01864	0.06638	0.1999	0.1413	2.2451	0.07414	0.02846	0.04386

TABLE VIB
THE SIXTEEN WAVELENGTH FTIR VALUE OF THREE FOOD FAT SAMPLES OF NHFA.HFA. AND NHFB INFRARED REGION (1400 – 650 CM^{-1}).

	Finger Print							
	1236	1216.3	1178	1141	1116.6	1098.4	1082.7	965.1
NHFA	0.07248	0.06602	0.1198	0.1385	0.09717	0.09357	0.06921	0.03024
HA	0.0756	0.06838	0.1235	0.1417	0.09756	0.09682	0.07295	0.03287
NHFB	0.07268	0.06613	0.1204	0.11397	0.09739	0.09391	0.06946	0.02982

E. Food Samples Statistical Analysis

FTIR Spectrometer was used to acquire the full spectrum in the mid infrared region (400-4000 cm^{-1}). The whole FTIR spectrum corresponded to the stretching of the functional groups present in the fat. The graph shows the average spectrum of five repetition each for PF, CF, BF, LF, PO, Mix PF-CF (9 fat), Mix PF-PO (9 fat), NHFA, HA and NHFB.

The twenty six fat samples were then injected into the FTIR device. Each fat was injected five times. The values reported were the average values of five replicates. Data obtained from FTIR was further processed using infrared reader software. The graphical display of the sixteen fats are shown in the following Fig. 9.

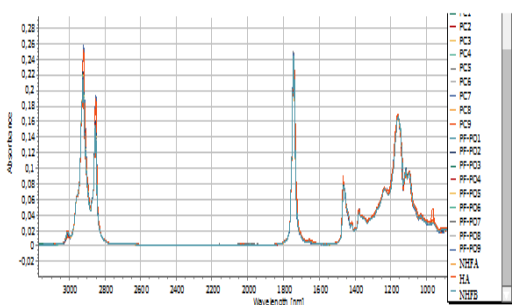


Fig. 9 FTIR spectra of lipid fraction extracted from twenty-six fat samples averaged of PF,CF,BF,LF,PO. Mix PF-CF. Mix PF-PO. NHFA,HA, and NHFB in infrared region (4.000 – 650 cm-1).

Sixteen wavelength of interest were identified which include four wavelength in the functional group region and twelve wavelength in the fingerprint region. The above values show the position of each fat at the wavelength above so that their positions could be compared with each other. The values were determined directly using software. The sample values at the sixteen wavelengths are summarized in the following Table VIIA and VIIB.

TABLE VIIA
THE SIXTEEN WAVELENGTH FTIR VALUE OF TWENTYSIX FAT SAMPLES
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
PC1	0.01921	0.06721	0.1976	0.1395	0.2491	0.07432	0.02878	0.04451
PC2	0.01915	0.06727	0.198	0.1399	0.2494	0.07462	0.02901	0.04462
PC3	0.01912	0.06709	0.1981	0.14	0.2492	0.07481	0.0292	0.04483
PC4	0.01904	0.06701	0.1983	0.1401	0.2493	0.07477	0.02911	0.04465
PC5	0.01915	0.06706	0.1987	0.1405	0.2493	0.07489	0.02915	0.04471
PC6	0.01896	0.06676	0.199	0.1407	0.2489	0.07481	0.02906	0.0445
PC7	0.01916	0.06697	0.1993	0.141	0.2489	0.0747	0.02896	0.04431
PC8	0.01921	0.06736	0.1998	0.1412	0.2468	0.07477	0.0289	0.04449
PC9	0.01914	0.06745	0.2004	0.1416	0.2487	0.07487	0.02878	0.04431
PF-PO1	0.01578	0.06551	0.2084	0.1481	0.2373	0.07656	0.02894	0.04525
PF-PO2	0.01601	0.06671	0.2091	0.1483	0.2388	0.07672	0.02894	0.0452
PF-PO3	0.01604	0.0657	0.2083	0.148	0.2388	0.0767	0.02913	0.0453
PF-PO4	0.0164	0.06602	0.2071	0.147	0.2413	0.07624	0.02884	0.04495
PF-PO5	0.01704	0.06633	0.2061	0.1461	0.2454	0.07599	0.02891	0.04478
PF-PO6	0.01715	0.06609	0.2053	0.1456	0.2457	0.07588	0.02886	0.0447
PF-PO7	0.01799	0.06646	0.2037	0.1443	0.2473	0.07579	0.02922	0.04477
PF-PO8	0.01832	0.06637	0.2021	0.1431	0.248	0.07526	0.0289	0.0444
PF-PO9	0.01866	0.06656	0.2012	0.1423	0.2484	0.07487	0.0287	0.04417
NHFA	0.01835	0.06569	0.1995	0.1414	1.602	0.07425	0.02842	0.04374
HA	0.02001	0.06712	0.1907	0.1345	1.686	0.07378	0.03008	0.04532
NHFB	0.01864	0.06638	0.1999	0.1413	0.2451	0.07414	0.02846	0.04386

TABLE VIIB
THE SIXTEEN WAVELENGTH FTIR VALUE OF TWENTYSIX FAT SAMPLES
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
PC1	0.07378	0.06648	0.1219	0.1419	0.09822	0.09469	0.07132	0.03014
PC2	0.07389	0.06652	0.122	0.1421	0.0982	0.09489	0.07116	0.03044
PC3	0.07391	0.06678	0.1221	0.1422	0.09865	0.09533	0.07156	0.03085
PC4	0.07379	0.06662	0.1221	0.142	0.09859	0.09518	0.07125	0.03053
PC5	0.07373	0.06668	0.1219	0.1419	0.09856	0.09516	0.07111	0.03066
PC6	0.07348	0.06654	0.1217	0.1416	0.09831	0.09505	0.07064	0.03049
PC7	0.07337	0.06635	0.1214	0.1412	0.09821	0.09482	0.0704	0.03018
PC8	0.0733	0.06637	0.1215	0.1405	0.09784	0.09487	0.07039	0.03012
PC9	0.07317	0.06627	0.1212	0.1409	0.09797	0.09482	0.07014	0.0301
PF-PO1	0.07373	0.06702	0.1226	0.1364	0.1002	0.09291	0.06851	0.02958
PF-PO2	0.07372	0.06705	0.1227	0.1369	0.1001	0.0932	0.06869	0.0297
PF-PO3	0.0738	0.06709	0.1228	0.1369	0.1002	0.09342	0.06892	0.0297
PF-PO4	0.07361	0.06684	0.1225	0.1378	0.09968	0.09353	0.06882	0.02957
PF-PO5	0.07351	0.06672	0.1222	0.1393	0.09969	0.09416	0.06925	0.02965
PF-PO6	0.07341	0.06678	0.1219	0.1395	0.09944	0.09417	0.06951	0.0297
PF-PO7	0.07352	0.06679	0.122	0.1402	0.09922	0.09474	0.07009	0.03033
PF-PO8	0.07322	0.06641	0.1215	0.1404	0.09849	0.09456	0.06991	0.03013
PF-PO9	0.07303	0.06623	0.1211	0.1407	0.09814	0.09469	0.07003	0.02966
NHFA	0.07248	0.06602	0.1198	0.1385	0.09717	0.09357	0.06921	0.03024
HA	0.0756	0.06838	0.1235	0.1417	0.09756	0.09682	0.07295	0.03287
NHFB	0.07268	0.06613	0.1204	0.1397	0.09739	0.09391	0.06946	0.02982

All the values in Table VIIA and VIIB were entered in the reader software to display the scatter plot image as a whole. The resulting score plot is shown in the following Fig. 10.

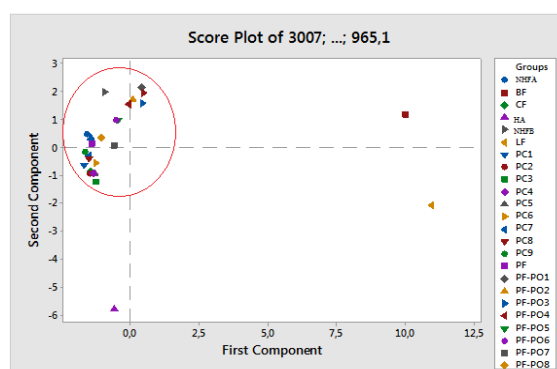


Fig. 10 Score plot of twenty six samples at sixteen wavelengths (3007 to 965.1 nm)

Fig. 10 shows that the wavelength for the three food fat samples NHFA, HA and NHFB were located very close to pig fat, chicken fat, palm oil and the pig fat mixtures; making it difficult to identify these fats. However, specific wavelength 1236 nm and 3007 nm can distinguish these fats.

TABLE VIII
THE TWO WAVELENGTH (3007 AND 1236) FTIR VALUE OF TWENTY SIX FAT
SAMPLES INFRARED REGION (4.000 – 650 CM-1).

Groups	3007	1236
BF	0.01158	0.07361
CF	0.0192	0.07394
LF	0.01173	0.07417
PF	0.01891	0.07307
PO	0.01521	0.07387
PC1	0.01921	0.07378
PC2	0.01915	0.07389
PC3	0.01912	0.07391
PC4	0.01904	0.07379
PC5	0.01915	0.07373
PC6	0.01896	0.07348
PC7	0.01916	0.07337
PC8	0.01921	0.0733
PC9	0.01914	0.07317
PF-PO1	0.01578	0.07373
PF-PO2	0.01601	0.07372
PF-PO3	0.01604	0.0738
PF-PO4	0.0164	0.07361
PF-PO5	0.01704	0.07351
PF-PO6	0.01715	0.07341
PF-PO7	0.01799	0.07352
PF-PO8	0.01832	0.07322
PF-PO9	0.01866	0.07303
NHFA	0.01835	0.07248
HA	0.02001	0.0756
NHFB	0.01864	0.07268

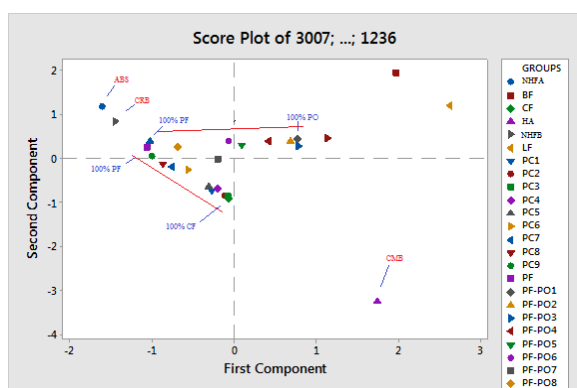


Fig. 11 Score plot of sixteen wavelength (3007 to 965.1) of twenty-six samples

Fig. 11 shows the plot scores of twenty-six fats at two wavelength along the graph (1236 and 3007 nm). NHA, H and NHB were identified at visible distances from other fat samples making them easier to identify. Therefore using these two wavelength for identification would result in good resolution between the three food samples.

Wavelength for the first two samples NHFA and NHFB were located very close to PF (Pig Fat). indicating that NHFA and NHFB samples contained pork fat; wavelength for HA was located very close to CF. indicating that H sample possibly contains chicken fat.

IV. CONCLUSIONS

At wavelength 1236 and 3007 nm along the spectrum; NHFA, HA and NHFB fat samples were easily identified at visibly good distance compared to other fat samples. The first two samples; NHFA and NHFB that were located very close to PF (Pig Fat) indicating that NHFA and NHFB samples contained pork fat while HA was located closer to CF, indicating that the sample possibly contained chicken fat. To this end, FTIR coupled with PCA has been shown to be a powerful tool to detect adulteration in meat products and as such can be recommended for authentication purposes.

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REFERENCES

- [1] A. Brangule, I. Skadiņš, A. Reinis, K.A. Gross and J. Kroča, "In Vitro characterization perspectives using Fourier Transform Infrared Photoacoustic Spectroscopy (FTIR PAS) and Diffuse Reflectance Infrared Spectroscopy (DRIFT)," *Key Engineering Materials*, vol. 758, pp. 273-277, 2017.
- [2] Y.B. Che Man, M.E.S.Mirghani, "Detection of lard mixed with body fats of chicken, lamb and cow by Fourier Transform Infrared Spectroscopy," *Journal of American Oil Chemist Society*, vol. 8, no.7, pp. 753-761, 2001.
- [3] Y.B. Che Man, A. Rohman, "The optimization of FTIR spectroscopy combined with partial least square for analysis of animal fats in quaternary mixtures," *Spectroscopy*, vol. 25, pp. 169-176, 2011.
- [4] C. Constantin, "Principal Component Analysis – a powerful tool in computing marketing information," *Bulletin of the Transilvania University of Braşov Series V: Economic Sciences*, vol.7, no.56, 2014.
- [5] N.A. Fadzilliah, Y.B. Che Man, M.A. Jamaludin, A.S Rahman, H.A. Al-Kahtani, "Halal Food Issues from Islamic and Modern Science Perspectives," *2nd International Conference on Humanities, Historical and Social Sciences IPEDR*, vol. 17, 2011.
- [6] K.D. Hargin, "Authenticity issues in meat and meat products," *Meat Science*, vol. 43, no. 96, pp. 277- 289, 1996
- [7] M.A. Jamaludin, C.W.J.W.M. Radzi, "Teori Istihalah menurut perspektif Islam dan sains: aplikasi terhadap beberapa penghasilan produk makanan," *Jurnal Syariah*, vol.17, pp. 169- 194, 2009.
- [8] M. Montowska, E. Pospiech, "Authenticity determination of meat and meat products on the protein and DNA basis," *Food Review International*, vol.27, pp. 84-100, 2014.
- [9] A. Rohman, Y.B. Che Man, "FTIR spectroscopy combined with chemometrics for analysis of lard in the mixtures with body fats of lamb, cow, and chicken," *J. Food Lipids*, vol.16, pp.618-628, 2009.
- [10] S. Yusof, A. Abd Rahim, J. Jalil, *Perkembangan dalam ingredien makanan: cabaran Malaysia dalam menangani isu Halal*, in *Penjenamaan Halal Satu Paradigma Baru*. ed. Mohd Noorizzuddin Nooh. Bangi: USIM. 99-120. 2007
- [11] C.B.Y. Cordella, "PCA: The Basic Building Block of Chemometrics," in *Analytical Chemistry*, Ed. I.S. Krull, InTech Open, 2012.
- [12] I.T. Jolliffe, J. Cadima, "Principal component analysis: a review and recent developments," *Philos Trans A Math Phys Eng Sci.*, vol. 374, no. 2065, Apr. 2013.