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Prediction of biomarker peak of infrared spectra between pork fat and chicken meat fat using fourier transform infrared spectroscopy technique and clotter plot method

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ABSTRACT

The increasingly widespread contamination of chicken meat by pork requires a method to identify the presence of these contaminants quickly and cheaply. Apart from analyzing the spectrum of various pure animal fats, this research also aims to vary the concentration of pork fat and chicken fat to know the FTIR peak spectrum, which will change with changes in concentration. The presence of spectral peaks that change can be identified due to changes in pork fat concentration, which also shows a typical peak spectrum position in pork fat. Variations in the concentration of pork fat contaminants in chicken fat consist of the ratio of the mixture of pork fat (PF) and chicken fat (CF), namely 90:10 (PC1), 80:20 (PC2), 70:30 (PC3), 60:40 (PC4), 50:50 (PC5), 40:60 (PC6), 30:70 (PC7), 20:80 (PC8), and 10:90 (PC9). A scatter plot, a graph usually used to see the relationship pattern between 2 variables, is employed in this research to visualize the changes in spectral peaks with varying concentrations of pork fat in chicken fat. The data scale must be an interval and ratio scale to use a scatter plot. Biomarker wavelengths were identified from the spectra of four animal fats and palm oil at positions 2948.9 and 3007 cm^{-1}, separated by four animal fats and palm oil at a certain distance, thus indicating that these wavelengths could be used to identify non-halal samples.

Keywords: Chicken, Clotter plot, Fat, Infrared, Meat, Pork, Spectra

INTRODUCTION

As a form of implementing worship, one of which is to create halal food for Muslims, especially in identifying the presence of pork contaminants in food products, a research method was carried out to test the presence of pork contaminants. Pork contamination in various products needs to continue to be developed. Testing for pork contaminants in a product is one type of material testing that will immediately clarify legal conclusions if the substance is present (Visciano & Schirone, 2020). This is the most apparent problem in the field of chemistry, and it is the duty and responsibility of Muslim chemists to help people obtain legal certainty regarding the products they use (Kovac, 2015). Food components containing pork in food ingredients and products can be identified through fat, protein, and Deoxyribonucleic Acid (DNA) (Yeh et al., 2024; Zia et al., 2020). A method developed to test the presence of pork contaminants in various products is identifying the presence of fat (Ling et al., 2024) because pork fat is usually present in small quantities with the meat. Pork fat content testing can be carried out using the Gas Chromatography (GC) method (Hewavitharana et al., 2020), Gas Chromatography-Mass Spectrometry (GCMS) (Fiehn, 2016), from this research, it was identified that there were only typical fatty acids and ion mass fragments present in pork fat samples which are not present in beef fat. However, this method is still relatively complicated to prepare and quite expensive. In contrast, the FTIR method, developed in 2003, offers a practical, cost-effective solution. It has found specific extended C-H vibrations in pork fat samples that differ from other animal fats (Fahelelbom et al., 2022). This FTIR method is a fast, simple, easy, and relatively cheap identification method and can even be tested directly without going through a complicated wet chemical preparation stage. This research won a Gold Medal at the 34th International Exhibition of Inventions, New Techniques and

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Products of Geneva, Geneva, Switzerland, 5 – 9 April 2006, further validating its practicality and cost-effectiveness.

The presence of unique fats in pork not found in other animal meats suggests the possibility of unique molecular vibrational properties of pork fat not found in other animal fats (Chernukha et al., 2023; Schumacher et al., 2022). In general, pork has a thick layer of fat with quite fine fibers. However, it is challenging to differentiate pork fat from chicken fat; they are very similar, especially when mixed. For this reason, this research will investigate the spectral biomarkers of pork fat against chicken fat based on the Fourier Transform Infrared Spectroscopy (FTIR) technique and clotter plot method. The research results include comparing the spectral patterns of pork fat and chicken fat to determine the spectral biomarkers of pork fat and the development of an infrared sensor based on software to detect pork fat. Scatter graph analysis in research using Minitab software. A scatter plot is a graph usually used to see the relationship pattern between 2 variables. The data scale must be an interval and ratio scale to use a scatter plot.

RESEARCH METHOD

Materials

In this study, the objects of research were raw pork fat and chicken fat, both in a pure state as a control and in a mixed state with several variations in concentration. Variations in the concentration of pork fat contaminants in chicken fat consist of: the ratio of the mixture of pork fat (PF) and chicken fat (CF) is 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 90% (in weight percentage). Making concentration variations aims to identify FTIR spectral peaks that will change with changes in concentration. The presence of spectral peaks that change can be identified due to changes in pork fat concentration, which also shows the peak spectrum position typical of pork fat. Theoretical and literature studies identify the types of molecular vibrations indicated by changing spectral peaks. Variations in the concentration of the mixture of chicken and pork fat were made to a value small enough to determine the detection limit of the method used, as well as a comparison in the fat analysis of beef, goat meat, and palm oil.

Methods

Samples from pigs, chickens, goats, and farm animals have been organized for analysis. The meat is received from neighborhood slaughterhouses in markets around Jakarta, Indonesia. Preparation starts by washing the pattern well and using distilled water to prevent infection on the beef pattern's floor. Then, the beef samples were decreased to a small size (1 cm x 1 cm) and saved at -20 ºC till used. Animal meat (pork, chicken, beef, lamb) becomes made via way means of processing animal adipose tissue consistent with strategies formerly pronounced via way means by Rohman et al. (2020) in this process. The meat is reduced into small pieces. Mixture and melt at (90 – 100)°C for two hours inside the oven. The melted fats are filtered via a triple-folded muslin cloth, dried using anhydrous Na_2SO_4, and centrifuged at 3000 rpm for 20 minutes. A layer of fats is poured off, shaken nicely, and centrifuged once earlier than filtering via Whatman clear-out paper containing anhydrous sodium sulfate to do away with residual water. The oil became organized at that time.

Data analysis

1. Analysis using FTIR spectroscopy

A Nicolet iS50 FTIR spectrometer is used to gain the overall spectrum inside the mid-infrared region (400 – 4000 cm^{-1}). The scan range becomes 32 with a decision of (4 cm^{-1}). Measurements are calibrated towards a clean background. All FTIR spectra are related to the stretching of practical corporations and fingerprint corporations gift inside the fats samples (Gao et al., 2020).

2. Exploratory data analysis: Scatter plot

Scatter graph analysis in research using Minitab software. A scatter plot is a graph usually used to see the relationship pattern between 2 variables. The data scale must be an interval and ratio scale to use a scatter plot. Apart from analyzing the spectrum of various pure animal fats, this research also varied the concentration of pork fat and chicken fat to know the FTIR peak spectrum, which would

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change with changes in concentration. The presence of spectral peaks that change can be identified due to changes in pork fat concentration, which also shows the peak spectrum position typical of pork fat. Variations in the concentration of pork fat contaminants in chicken fat consist of the ratio of the mixture of pork fat (PF) and chicken fat (CF), namely 90% pork fat and 10% chicken fat (PC1), 80% pork fat and 20% pork fat. % chicken fat (PC2), 70% lard and 30% chicken fat (PC3), 60% lard and 40% chicken fat (PC4), 50% lard and 50% chicken fat (PC5), 40% lard and 60% chicken fat (PC6), 30% pork fat and 70% chicken fat (PC7), 20% pork fat and 80% chicken fat (PC8), and 10% pork fat and 90% chicken fat (PC9) (based on heavy). The spectrum analysis was carried out using peak spectrum analysis by comparing the absorption of various fats at one IR wavelength peak.

RESULT AND DISCUSSION

The absorbance of each pure fat

An FTIR spectrometer was used to achieve the spectrum inside the mid-infrared region (400 – 4000 cm^{-1}). The 4 animal fats and palm oil are injected into the FTIR. Each fat is injected 5 times; The values mentioned are the shared values of the 5 tests. All FTIR spectra acquired correspond to the variety of purposeful companies found in fats. Table 1 indicates the common 5 spectral readings for beef fats, chicken fats, pork fats, lamb fats, and palm oil. Data acquired from FTIR also processed the usage of infrared reader software. The spectrum inside the shape of transmittance of all samples usually has styles that can be just like every other, so the variations can not be diagnosed with certainty. The spectrum converted in absorption shape offers nearly identical samples for all samples. Table 1 shows the absorbance spectrum results of five types of fats and oils.

Table 1. Absorbance spectrum of pork fat (PF), chicken fat (CF), beef fat (BF), lamb fat (LF), and palm oil (PO).

	Groups (cm^{-1})	PF (A)	CF (A)	BF (A)	LF (A)	PO (A)
Functional groups	3007	0.01891	0.0192	0.01158	0.01173	0.01521
	2948.9	0.06633	0.06706	0.06344	0.06336	0.06598
	2918	0.1992	0.1967	0.259	0.2529	0.211
	2850	0.1413	0.1392	0.1943	0.1887	0.1505
Fingerprint	1743.1	0.2461	0.2462	0.2398	0.2407	0.2414
	1466	0.07467	0.07448	0.09155	0.08989	0.07704
	1416.5	0.02884	0.02901	0.03095	0.03124	0.02896
	1377.7	0.04415	0.04472	0.04852	0.04811	0.04531
	1236	0.07307	0.07394	0.07361	0.07417	0.07387
	1216.3	0.06632	0.06657	0.07199	0.07258	0.06715
	1178	0.1208	0.122	0.1374	0.1361	0.1229
	1141	0.1402	0.1412	0.128	0.128	0.1373
	1116.6	0.09793	0.0981	0.097	0,09608	0.1009
	1098.4	0.09469	0.09469	0.09407	0.09593	0.09335
	1082.7	0.07031	0.0715	0.06134	0.06097	0.06858
	965.1	0.03025	0.0306	0.03136	0.04871	0.02939

A comparison of statistical descriptions for the five types of fats and oils can be seen in Table 2 below. Table 2 shows the mean values and standard deviations of the five types of fats and oils. The mean and standard deviation values for pork fat and chicken fat are identical: a mean of 0.095 and a standard deviation of 0.063; this differs from those for beef fat, lamb fat, and palm oil. The comparison of absorbance values for the five types of fats and oils can also be seen using scatter plots in Figure 1 below.

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Table 2. Descriptive Statistics of pork fat (PF), chicken fat (CF), beef fat (BF), lamb fat (LF), and palm oil (PO).

	PF	CF	BF	LF	PO
Valid	16	16	16	16	16
Missing	0	0	0	0	0
Mean	0.095	0.095	0.102	0.102	0.096
Std. Deviation	0.063	0.062	0.073	0.071	0.064
Minimum	0.019	0.019	0.012	0.012	0.015
Maximum	0.246	0.246	0.259	0.253	0.241

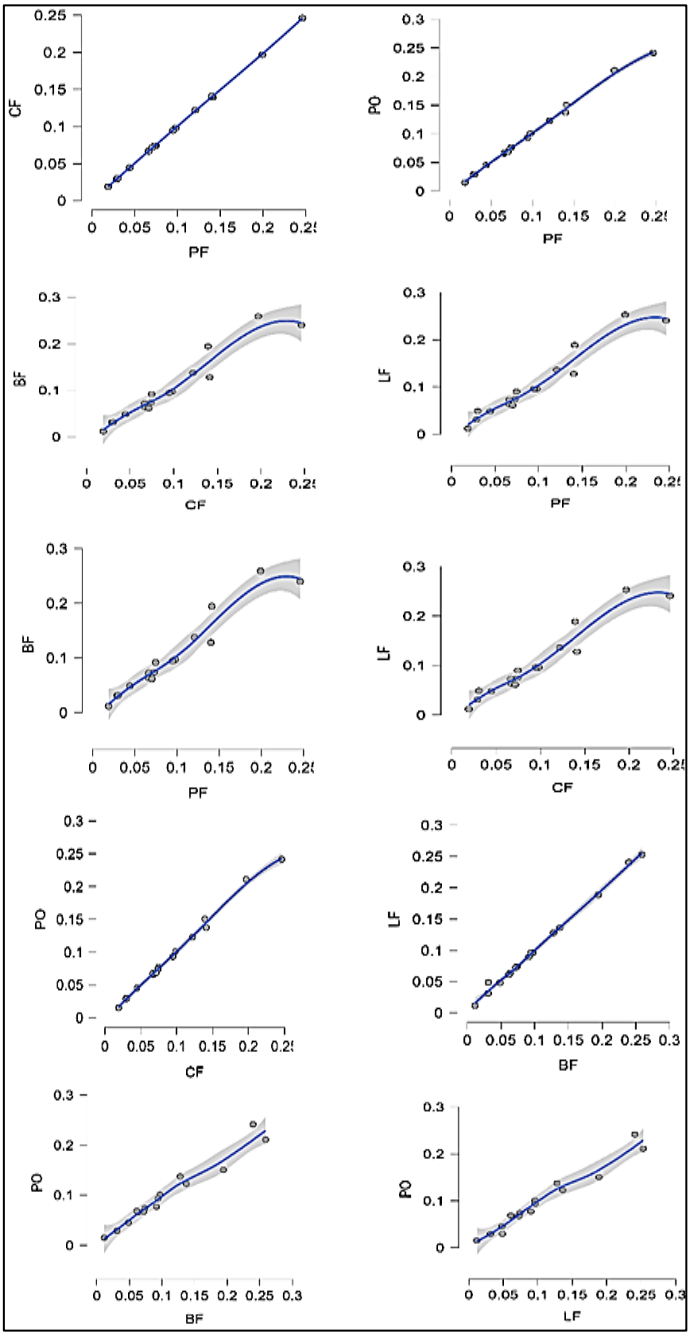

Figure 1. Comparison of absorbance values for five types of fats and oils using the scatter plots method of pork fat (PF), chicken fat (CF), beef fat (BF), lamb fat (LF), and palm oil (PO).

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Figure 1. shows that the absorbance spectrum values between PF and CF are identical. This indicates that the types of pork fat and chicken fat are almost one hundred percent the same. This result is similar to the research results from Saputra et al. (2018). In the following analysis, we focused on looking at the differences in the absorbance spectra of Pig fat and chicken fat in more detail at the absorbance peaks of the spectrum.

Absorbance of mixed variations of pork fat and chicken fat

This study tested a mixture of 2 animal fats: pork fat and chicken fat. The aim of testing a mixture of animal fats is to find out how much influence the concentration of each fat has on the absorption position of pure fat. Variations in the concentration of pork fat contaminants in chicken fat consist of the ratio of the mixture of pork fat (PF) and chicken fat (CF), namely 90% pork fat and 10% chicken fat (PC1), 80% pork fat and 20% pork fat. % chicken fat (PC2), 70% lard and 30% chicken fat (PC3), 60% lard and 40% chicken fat (PC4), 50% lard and 50% chicken fat (PC5), 40% lard and 60% chicken fat (PC6), 30% pork fat and 70% chicken fat (PC7), 20% pork fat and 80% chicken fat (PC8), and 10% pork fat and 90% chicken fat (PC9) (based on heavy). Making concentration variations aims to identify FTIR spectral peaks that will change with changes in concentration. The presence of spectral peaks that change can be identified due to changes in pork fat concentration, which also shows the peak spectrum position typical of pork fat. Table 2 shows the average five spectral readings for a pork and chicken fat mixture with varying concentration ratios. Data obtained from FTIR is further processed using infrared reader software.

Table 2. shows an average of five spectral readings each for a mixture of pork fat and chicken fat (PC) with varying concentration comparisons.

	Groups	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Functional groups	3007	0.01921	0.01915	0.01912	0.01904	0.01915	0.01896	0.01916	0.01921	0.01914
	2948.9	0.06721	0.06727	0.06709	0.06701	0.06706	0.06676	0.06697	0.06736	0.06745
	2918	0.1976	0.198	0.1981	0.1983	0.1987	0.199	0.1993	0.1998	0.2004
	2850	0.1395	0.1399	0.14	0.1401	0.1405	0.1407	0.141	0.1412	0.1416
Fingerprint	1743.1	0.2491	0.2494	0.2492	0.2493	0.2493	0.2489	0.2489	0.2468	0.2487
	1466	0.07432	0.07462	0.07481	0.07477	0.07489	0.07481	0.0747	0.07477	0.07487
	1416.5	0.02878	0.02901	0.0292	0.02911	0.02915	0.02906	0.02896	0.0289	0.02878
	1377.7	0.04451	0.04462	0.04483	0.04465	0.04471	0.0445	0.04431	0.04449	0.04431
	1236	0.07378	0.07389	0.07391	0.07379	0.07373	0.07348	0.07337	0.0733	0.07317
	1216.3	0.06648	0.06652	0.06678	0.06662	0.06668	0.06654	0.06635	0.06637	0.06627
	1178	0.1219	0.122	0.1221	0.1221	0.1219	0.1217	0.1214	0.1215	0.1212
	1141	0.1419	0.1421	0.1422	0.142	0.1419	0.1416	0.1412	0.1405	0.1409
	1116.6	0.09822	0.0982	0.09865	0.09859	0.09856	0.09831	0.09821	0.09784	0.09797
	1098.4	0.09469	0.09489	0.09533	0.09518	0.09516	0.09505	0.09482	0.09487	0.09482
	1082.7	0.07132	0.07116	0.07156	0.07125	0.07111	0.07064	0.0704	0.07039	0.07014
	965.1	0.03014	0.03044	0.03085	0.03053	0.03066	0.03049	0.03018	0.03012	0.0301

Exploratory data analysis: Scatter plot per one IR wavelength peak

The peak wavelengths of IR absorption analyzed for absorbance comparison are 3007 nm, 2948.9 nm, 2918 nm, 2850 nm, 1466 nm, 1377.7 nm, 1216.3 nm, and 1178 nm.

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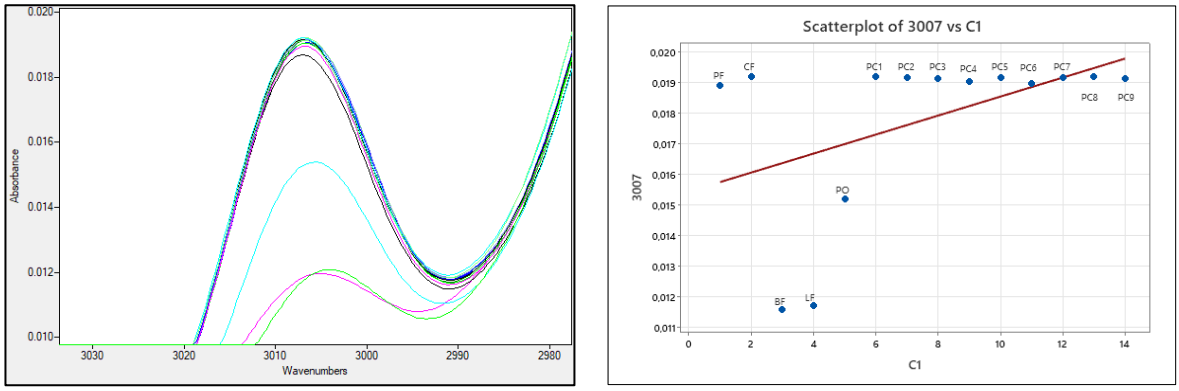

(a) (b)

Figure 2. FTIR spectra (a) and scatter plot (b) of fat extracted from five animal samples of lard (PF), chicken fat (CF), beef fat (BF), lamb fat (LF), and palm oil (PO), and mixed variations of lard and chicken fat at wavelength 3007 cm^{-1}.

The sharp C-H band at wave number 3007 cm^{-1} indicates the presence of an unsaturated group. This is strengthened by a weak but sharp band near wave number 1654 cm^{-1} caused by the C=C group. The presence of solid absorption at wave numbers 2925 and 2854 cm^{-1} is characteristic of the absorption of alkyl groups by Csp 3 -H stretching vibrations, reinforced by the presence of absorption at wave numbers 1462 cm^{-1}, which indicates the C-H bending vibration of the methylene group (-CH_2-). Based on the Spectral Database (SDBS) IR spectra database of linoleic acid, the unsaturated group -C=O ester. It can be seen that the spectral pattern of pork fat resembles the IR spectral pattern of linoleic acid, which is an unsaturated fatty acid that contains two double bonds or is included in the polyunsaturated fatty acids (PUFA) group.

Figure 2. shows that the two fat spectrums appear to have similar spectrum patterns because these two spectrums are typical spectra for edible oil fats in general. However, the absorption pattern in the 3400 cm^{-1} area for the pork fat sample shows a relatively higher peak when compared to the chicken fat sample (Parrini et al., 2023), with a frequency range of 3006 – 3000 cm^{-1}. Frequencies in 3007 cm^{-1} can be attributed to -C=CH (cis double bond stretching) and can be attributed to monounsaturated fatty acids (MUFA) (Saito et al., 2022) frequency range 1650 – 1645 cm^{-1}. The C=O organization of triglycerides shows a stretching band at approximately 1744 cm^{-1}. The C=C stretching mode of nonconjugated olefins typically exhibits moderate to slight absorption between 1667 and 1640 cm^{-1}. Unsubstituted trans-olefins absorb at 1670 cm^{-1}, but the band may be very sensitive or absent. Unsubstituted cis-olefins absorb around 1650 cm^{-1}, and the absorption in this band is more vital than that of trans-olefins. These bands can be attributed to the C=C stretching vibrations of the disubstituted cis-C=C of the acyl structures of oleic and linoleic acids (Pielesz et al., 2023) with a frequency range of 1380 – 1360 cm^{-1}. Frequencies in 1400 – 1000 cm^{-1} were the most difficult to allocate. At about 1464 cm^{-1}, all spectra showed scissor bands of bending vibrations of the methylene structure. Small bands that were difficult to identify were formed in all samples around 1400 cm^{-1} and 1377 cm^{-1}. This is thought to be due to the symmetric bond vibration of the methyl structure (Piwowarczyk et al., 2019), with a Frequency range of 1230 – 1228 cm^{-1}. In this range, slight changes are observed within the peak height in the frequency range 1200 – 1250 cm^{-1}. The twisting and wobbling vibrations of CH_2 units worldwide are between 1250 and 1150 cm^{-1}, and the individual bands generally stop acting as methylene scissors (Jordanov et al., 2003). Frequency range 1119 – 1096 cm^{-1} At this frequency, Harbrad showed overlapping peaks with maxima at 1098.69 cm^{-1} and 1116.88 cm^{-1}. These peaks were inversely proportional to the proportion of saturated acyl organization and oleic acyl groups, respectively (Dai et al., 2023).

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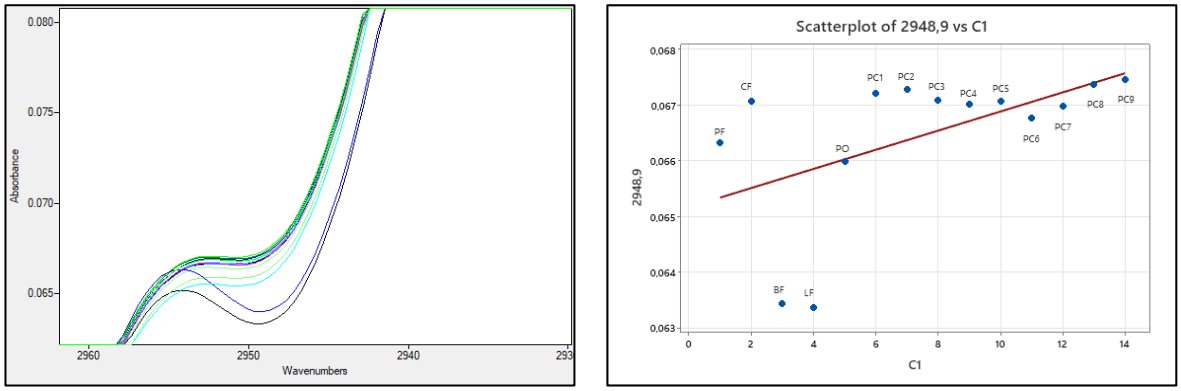
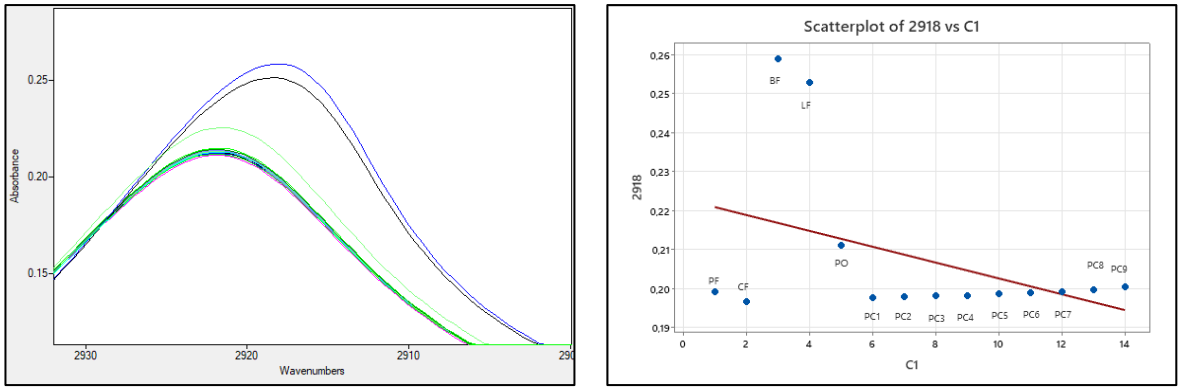


(a) (b)

Figure 3. FTIR spectra (a) and scatter plot (b) of fat extracted from five animal samples and mixed variations of lard and chicken fat at a wavelength of 2948.9 cm^{-1} of lard (PF), chicken fat (CF), beef fat (BF), lamb fat (LF), and palm oil (PO).

Based on these data, it can be seen that the FTIR spectrum of fat samples generally shows prominent differences in the stretching C-H absorption in the 3050 – 2800 wavenumber region, the absorption of carbonyl groups (O=C-H) from aldehydes in this region 1746 – 1744 cm^{-1} (Ellerbrock & Gerke, 2021), and absorption patterns in the fingerprint area 1000 – 900 cm^{-1}. Fundamental differences can be seen in the absorption spectrum in the 3010 – 3000, 1120 – 1095, and 968 – 966 cm^{-1} regions. The absorption pattern in the 3010 cm^{-1} area for the pork fat sample showed a relatively high peak compared to the other two fat samples (chicken and beef).



(a) (b)

Figure 4. FTIR spectra (a) and scatter plot (b) of fat extracted from five animal samples and mixed variations of lard and chicken fat at wavelength 2918 cm^{-1} of lard (PF), chicken fat (CF), beef fat (BF), lamb fat (LF), and palm oil (PO).

The high absorption peak of lard in this area indicates the stretching vibration of the C=C cis double bond. This is in line with the research results of Yi et al. (2023), where for pork fat samples, the content of polyunsaturated fatty acids or PUFA, such as linoleic acid and linolenic acid, was much more significant than monounsaturated fatty acids or MUFA. Furthermore, in the frequency area 1120 – 1095 cm^{-1}, the pork fat sample overlaps two peaks with maximum absorption at wave numbers 1118 and 1098 cm^{-1}. This differs from the spectrum patterns produced in beef fat and chicken fat samples, where the two samples do not overlap except for chicken fat, with a pattern almost similar to pork fat. This indicates the possibility of differences in the fatty acid profiles in the three samples. This is confirmed by research by Yi et al. (2023), which states that the overlap of the two wave number regions shows differences in the content of saturated and unsaturated fatty acids.

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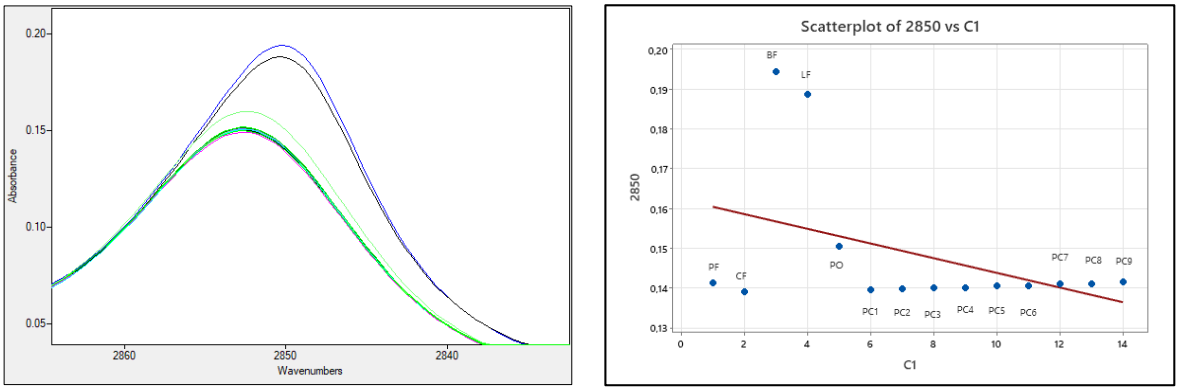
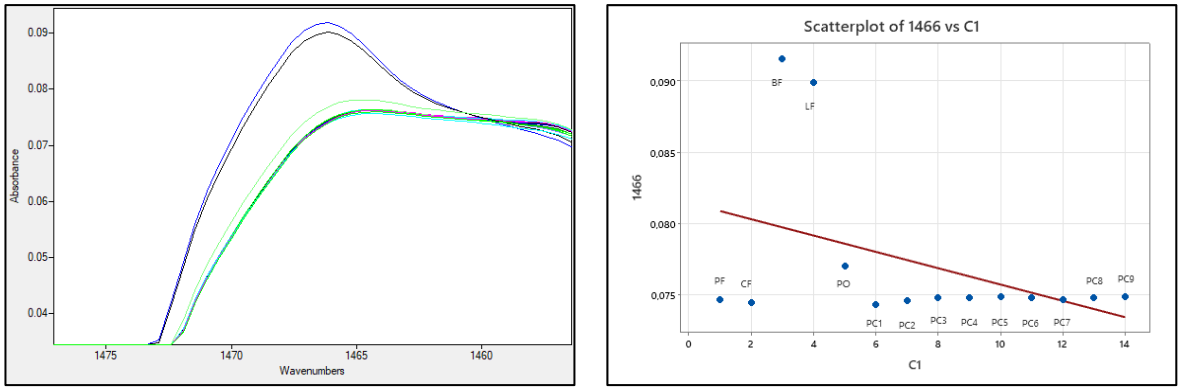


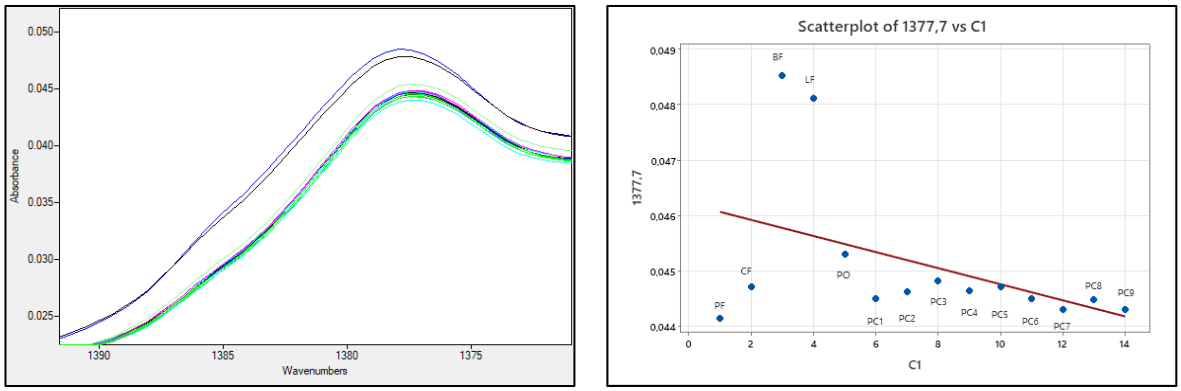
(a) (b)

Figure 5. FTIR spectra of fat extracted from five animal samples and mixed variations of lard and chicken fat at wavelength 2850 cm^{-1} of lard (PF), chicken fat (CF), beef fat (BF), lamb fat (LF), and palm oil (PO).



(a) (b)

Figure 6. FTIR spectra of fat extracted from five animal samples and mixed variations of lard and chicken fat at wavelengths of 1466 cm^{-1} of lard (PF), chicken fat (CF), beef fat (BF), lamb fat (LF), and palm oil (PO).



(a) (b)

Figure 7. FTIR spectra of fat extracted from five animal samples and mixed variations of lard and chicken fat at a wavelength of 1377.7 cm^{-1} of lard (PF), chicken fat (CF), beef fat (BF), lamb fat (LF), and palm oil (PO).

The scatterplot method has been proven to determine biomarker wavelengths from the spectra of four animal fats and palm oil (Anwardeen et al., 2023). Figure 1. shows that the absorbance spectrum values between PF and CF are identical. This indicates that the types of pork fat and chicken fat are almost one hundred percent the same. This result is similar to the research results from Saputra et al.

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(2018). Figure 2 until Figure 7. shows that the scatterplot method can show peaks in the absorbance spectrum between PF and CF. The absorbance spectrum value between pork fat and chicken fat, which is further away, is the absorbance spectrum value for palm oil, beef fat, and lamb fat in order is 3007 > 2948.9 > 2918 cm^{-1}. Three prominent frequencies are at points 3007, 2948.9, and 2918 cm^{-1}; the frequency at 3007 cm^{-1} is attributed to –C=CH (cis double bond stretching) and can be correlated with monounsaturated fatty acids (MUFA). Biomarker wavelengths were identified from the spectra of four animal fats and palm oil at positions 3007, 2948.9, and 2928 cm^{-1}, which were separated by four animal fats and palm oil at a certain distance, thus indicating that these wavelengths can be used to identify non-halal.

CONCLUSION

From the research results, it can be concluded that the scatterplot method has been proven to determine biomarker wavelengths from the spectra of four animal fats and palm oil. Biomarker wavelengths were identified from the spectra of four animal fats and palm oil at positions 3007, 2948.9, and 2918 cm^{-1}, separated by four animal fats and palm oil at certain distances, thus indicating that these wavelengths can be used to identify non-halal samples.

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