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Halal Verification of Imported Fish Pellets by FTIR-ATR Spectroscopy Approach and Multivariate Data Analysis

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ABSTRACT

Ensuring the authenticity of *halal* food is of paramount importance to Muslim consumers, as mandated by Islamic dietary laws. Recent reports have highlighted the widespread adulteration of food, pharmaceuticals, and aquaculture products with lard and porcine by-products, necessitating reliable and efficient methods for *halal* verification. This study aims to develop rapid authentication techniques employing multivariate data analysis (MVDA) of principal component analysis (PCA) for verifying *halal* status. The study utilizes FTIR-ATR for the analysis of oils extracted using a Soxhlet apparatus from animal sources such as beef, chicken, and pork, as well as from palm oil. Additionally, imported fish pellet samples from country of China, Taiwan and Japan were analysed. To achieve the *halal* verification, the FTIR-ATR spectra of the oils were scrutinized, focusing specifically on the fingerprint region between 1500 and 600 cm^{-1}. This region is known to contain unique spectral features that can differentiate between various types of oils. By applying PCA to the spectral data, distinct patterns corresponding to each type of oil were identified and differentiated. The results demonstrate that FTIR-ATR combined with MVDA provides a robust method for the rapid and accurate authentication of *halal* status in food products. This approach not only enhances the reliability of *halal* verification but also contributes to safeguarding consumer trust and compliance with dietary regulations. This research underscores the potential of FTIR-ATR spectroscopy combined with advanced chemometric techniques in ensuring the integrity of *halal* food imported products in the market of Muslim majority country.

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1. Introduction

*Halal i*s described by Islamic law as allowed and lawful, whereas "*haram*" denotes for prohibited. For Muslims, the consumption of non-*halal* foods such as pork and its derivatives are forbidden. *Halal* foods regardless must comply with specific guidelines outlined in Islamic law, which prohibit the consumption of certain items as aforementioned above. Additionally, the slaughtering process for *halal* meat must follow prescribed methods to ensure the animal's well-being and minimize suffering. However, the majority of terminology associated with *halal* products are related only to food, drink and consumer goods, including medicines and cosmetics. Furthermore, product adulteration has been brought about by new technologies in the manufacturing sector since certain goods may contain undeclared compounds that raise suspicions in the minds of certain consumers and non-*halal* elements. At the moment, the Islamic authorized parties employ various analytical techniques to detect and quantify the presence of potential non-*halal* components/contaminants such as HPLC which can separate and identify specific compounds based on their chemical properties of porcine-based materials or non-*halal* animal fats. Other analytical techniques, such as mass spectrometry, gas chromatography and DNA analysis, may also be employed to provide complimentary information on the composition and purity of the food product [1].

The aquaculture industry plays a vital role in global food security and economic development. Imported fish pellets represent a significant component of the aquafeed supply chain, catering to the growing demand for aquaculture products worldwide. The economic importance of this trade underscores the need for rigorous *halal* verification to ensure compliance with religious dietary laws and maintain consumer confidence in Muslim-majority markets. Certain regions, particularly those with significant Muslim populations, rely heavily on imported fish pellets to support their domestic aquaculture operations. The prevalence of imported aquafeeds in these markets heightens the necessity for *halal* verification to meet the religious and cultural requirements of the local consumer base. Failure to ensure *halal* compliance could potentially lead to market rejection and economic losses. The fish pellet is formulated to meet the comprehensive nutritional requirements of the fish, ensuring the support and maintenance of vital physiological processes. This includes promoting healthy development, facilitating successful reproduction and bolstering the immune system, regardless of the species-specific needs. For these reasons, a wide range of additives, including preservatives such as nitrites, antioxidants and enzymes, have been added to fish pellet formulation [2]. The aquaculture industry has expressed interest in phytogenic feed additives (PFA), derived from fruits, roots and leaves, available in both liquid and solid forms. Additionally, palm and seed oils, recognized for their safety and efficacy, are commonly incorporated into fish pellet formulations due to their ability to enhance feed intake and digestibility [3]. Owing to the aforementioned benefits, there is a growing interest in replacing these oils with lard. By adding lard to fish pellets has increased fish growth as it is a cost-effective source of fat as well as by adding small amount of lard increase the palatability of the pellets, encouraging fish to eat them more readily compared to other PFAs [4], but this substitution has caused concern among consumers, particularly vegetarians [5], the Jews [6] and the Muslims, when the manufacturers of fish feed make false claims and compromise the integrity of the feed. Next, there is a concern in regards of *halal* authenticity in food industry by which known as *al-Jallalah* (contaminated) animals which has been reported throughout newspaper in last decades. One example is that there is a case where Tilapia fish was fed by pig waste in Perak where the manufacturer's claiming that the action to feed their fish is to accelerate the growth of fish and can be marketed in short period of time in a year if not given such food [7].

From a scientific perspective, the *halal* verification protocols and analytical techniques utilized for fish pellets can be adapted and extended to a wide range of imported food products, both of

animal and plant origin. The core principles of ingredient analysis, manufacturing process evaluation and supply chain traceability remain relevant across diverse food matrices. Advanced analytical techniques, such as DNA analysis, chromatography and mass spectrometry, which are employed in the *halal* verification of fish pellets, find equal relevance in the analysis of other imported food products. These scientific methods can be adapted to detect and quantify the presence of non-*halal* ingredients or contaminants in a wide range of food matrices, providing valuable insights for *halal* certification and regulatory compliance. The analytical community has expressed interest in using infrared spectroscopy to evaluate fats and oils [8]. Conversely, infrared spectroscopy is a useful analytical method for assessing food and pharmaceutical items since it is a vibrational form of spectroscopy and provides rapid evaluation at a low cost. The infrared spectroscopy is the most extensively utilized technique for food analysis, particularly within the mid-infrared (4000 - 400 cm^{-1}) and near-infrared (14000 - 400 cm^{-1}) spectral ranges [9]. The FTIR-ATR approach in the meanwhile provides a rapid, non-destructive and highly accurate analytical technique for identifying and quantifying the presence of potential non-*halal* substances in food products. This method leverages the unique molecular vibrational signatures of different compounds, which are captured by the infrared absorption spectrum. Moreover, the key advantages of FTIR-ATR are its reliability in detecting and quantifying even trace amounts of non-*halal* ingredients or contaminants, such as porcine-derived materials or non-*halal* animal fats. Traditional methods may struggle to identify these components, particularly when present in complex food matrices or at low concentrations. The FTIR-ATR approach is also highly efficient, as it requires minimal sample preparation and analysis time compared to other analytical techniques like chromatography or mass spectrometry. This efficiency translates into cost savings and faster turnaround times for *halal* verification processes, benefiting both manufacturers and certification bodies [10].

Due to the ability that helps to identify specific molecules present in the food sample and the chemical composition of the food products, the FTIR spectroscopy is frequently used in conjunction with chemometrics for the examination of non-*halal* materials, such as pig derivatives and a variety of non-*halal* meats such wild boar, dog and rat meats. This is due to its benefits, especially in regard to the FTIR instrument's fingerprint analysis technique [11,12]. However, it is important to note that the reliability of FTIR-ATR is contingent on the availability of comprehensive spectral libraries and robust chemometric models for accurate identification and quantification of non-*halal* components. Ongoing research and collaboration among scientific experts, *halal* certification bodies and regulatory authorities are crucial in developing and validating these reference databases and analytical models. Additionally, while FTIR-ATR offers significant advantages in terms of speed and accuracy, it may not entirely replace traditional *halal* verification methods. A holistic approach that combines FTIR-ATR with ingredient analysis, documentation review and on-site inspections can provide a comprehensive and robust *halal* verification process, ensuring the utmost integrity of *halal* supply chains [9]. Herein, this research article presenting the verification of *halal* utilizing the FTIR-ATR instrumentation combining with the multivariate data analysis using oils extracted from imported fish pellets through determining at the fingerprint's region of the FTIR spectra.

2. Methodology

2.1 Materials

Adipose tissue samples from chicken, beef and pork, along with imported fish pellets originating from countries such as Taiwan, Japan and China, were collected from local pet shops in Muar, Johor, Malaysia and labelled as samples #1, #2 and #3. The analytical solvents used for fat extraction was petroleum ether (boiling range: 60 – 80 °C, R&M Chemicals), palm oil (analytical standard, Sigma-

Aldrich) and magnesium sulphate, $MgSO_4$ (analytical standard, R&M Chemicals). All chemicals used were analytical grade and used without further purification.

2.2 The Treatment of the Animal Adipose Tissue

All the animal (chicken, beef and pork) fatty part of the meat were manually trimmed and cut into smaller pieces using commercial cutter by 1 cm × 1 cm cube and were put into vacuum drying oven (Memmert, Germany) for drying at 80 °C of temperature, 0.32 bar of pressure for 24 h. The dried animal adipose tissues were collected and stored in commercial freezer (Sharp, Japan).

2.3 Extraction of Fat from Animal Adipose Tissue and Imported Fish Pellets Samples

All the fat from the chicken, beef, pork and the imported fish pellets were extracted according to the established methodology with minor modification [13]. In general, 20 g of the dried samples of meat (chicken, beef and pork) and the imported fish pellets were weighed and then ground into a fine powder using a commercial blender before being placed into a cellulose extraction 30 × 100 mm size thimble. Then, the top of the thimble was covered by cotton wool as to prevent the sample floating before inserted into the Soxhlet apparatus. The extraction process was done in 6h using petroleum ether as the solvent. The acquired extracts were combined with a spoonful of $MgSO_4$ to eliminate the moisture presence. Subsequently, the mixture was filtered through filter paper and the filtrate was subjected to evaporation using a rotary evaporator. The resulting oil was then transferred into vials for storage.

2.4 FTIR-ATR Measurement

The Nicolet iS5 spectrophotometer model (Thermo Scientific, USA) was used in the measurements. An ATR accessory equipped with diamond cell was used. All spectra were recorded within a range of 4000 – 600 cm^{-1} with 4 cm^{-1} resolution and 32 scans. Three replicate spectra were obtained from three independent experiments and the average spectrum was taken for further investigation. All measurements were performed in a dry atmosphere at room temperature (25±0.5 °C). A single beam spectrum was obtained for all samples. These spectrums were subtracted against a background air spectrum and the results were presented in transmittance units. All the samples' spectra were read in triplicate and averaged using the OMNIC operating software from the Thermo Nicolet.

2.5 Dataset Pre-Processing

The spectra were converted into comma-separated values (CVS) and imported to the dataset table in XLSTAT 2024 software [14]. Firstly, the functional region (4000 – 1501 cm^{-1}) and fingerprint region (1500 – 650 cm^{-1}) were separated. Then, discriminant analysis was carried out on the fingerprint region at 1000 – 650 cm^{-1} and 1500 – 1001 cm^{-1}, respectively. The most significant wavenumbers from these regions were determined as such 1037 spectrum data involving the wavenumbers and %transmittance for 1000 – 650 and 1500 – 1001 cm^{-1} regions. Subsequently, the Kaiser-Meyer-Olkin (KMO) test verified dataset adequacy before carrying out second discriminant analysis (DA) with the combined wavenumbers [15]. The most significant wavenumbers were selected from DA and proceeded with PCA to find the apportionment of wavenumber for all the animals, palm and extracted fish pellets oils.

2.6 The Kaiser-Meyer-Olkin (KMO) Test

The dataset was analysed for dataset adequacy by the KMO test. An adequate dataset determines the ability to generated model to extract latent variables from the dataset. In this study, the KMO test was employed at significant level (α) of 0.01. The calculated KMO was ranked as KMO < 0.5 = inadequate, 0.5 < KMO < 0.7 = mediocre, 0.7 < KMO < 0.8 = good, 0.8 < KMO < 0.9 = very good and KMO > 0.9 = excellent to indicate the dataset adequacy [16].

2.7 The Dataset Transformation

To ensure that the dataset followed a normal distribution before the PCA, the dataset normality was tested using Shapiro-Wilk test at α = 0.01. The dataset was transformed using standard deviation (n-1) methods.

2.8 The Principal Component Analysis (PCA)

The whole FTIR spectra was extracted its transmittance value to obtain dataset for PCA. The FTIR spectra at the combination wavenumber of 1500 – 1000 cm^{-1} and 1001 – 650 cm^{-1} at the fingerprint's region were chosen to build the PCA model because it can be used for clear difference for *halal* authentication purpose. Analysis of PCA was performed using XLSTAT software and the data were scaled using Pareto scaling technique prior to PCA analysis to maximize the variation. After Pareto scaling, the variables used for PCA model were more normally distributed shown by its Gaussian curve. The number of principal components (PCs) was optimized to obtain optimum differentiation among samples. The differentiation result of samples was observed using PCA score plot. Moreover, PCA model was evaluated using its R^2 and Q^2 value to justify the good of fitness and predictivity of the PCA model, respectively [17].

3. Results

3.1 The FTIR-ATR Spectra Analysis

The extracted oils from dried beef, chicken and pig meats have a similar physical appearance which is yellow colour whereas the palm oil is in white colours. The imported fish pellets are all from various country such as Taiwan, Japan and China and were labelled as sample #1, #2 and #3, respectively. The FTIR spectra of all oil samples showed a high degree of similarity, making it challenging to differentiate between them as showed in Figure 1. This similarity is a significant barrier in differentiating between the oils, especially in terms of distinguishing of animal-derived oils (beef, chicken and pig) and palm oil. This method allowed for a direct visual comparison, highlighting the overlapping absorption bands characteristic of all samples. The commonality in absorption bands among these oils emphasizes the challenge of discerning their origins solely based on FTIR spectroscopy [18].

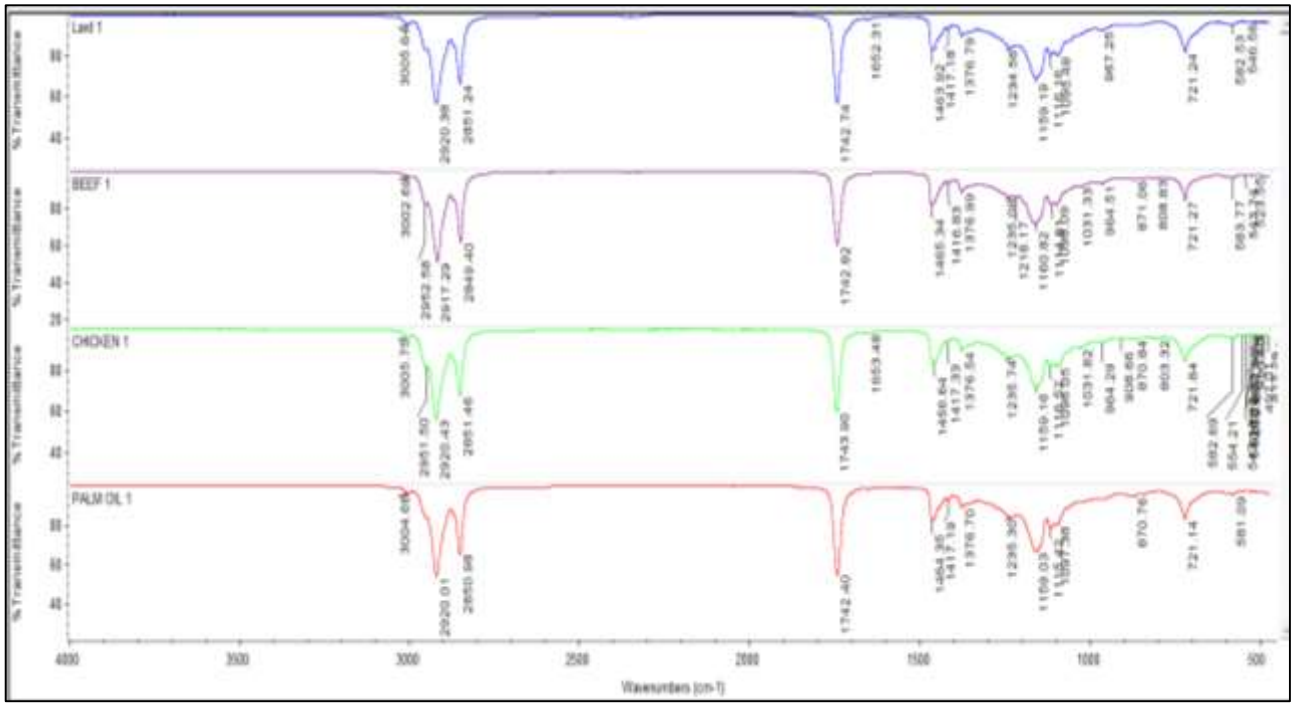

Fig. 1. The stacked of FTIR spectra comprising of animals (pork, beef, chicken) and palm oils

The common functional groups frequency responsible for IR absorption in the animal sources and palm oils are shown in Table 1 while the functional group frequency from the imported fish pellet oil samples is shown both in Tables 2 and 3. Nevertheless, the most prominent peak in the region of 2952 to 2849 cm^{-1} is due to C-H stretching mode of sp^2 and sp^3 hybridization between the carbon and the hydrogen atoms [19]. Noteworthy, the absorption of sp^2 C-H of the oils occurs at the higher frequency as compare with the sp^3 C-H absorption of the compound. This strength will result a larger vibrational force constant and a higher frequency of the vibration. The C=O stretching is observable in the region of 1700 cm^{-1}, while the wavenumber of 1400 – 1100 cm^{-1} is associated with C-O-C stretching and C-H bending [20].

Table 1
The frequencies and vibration modes for typical spectrum of fats and oils [18]

Frequency	Functional group	Types of vibrations	Remarks
3005	C–H	Symmetrical stretching	–CH=CH (*cis* olefin)
2922	C–H	Asymmetrical stretching	Aliphatic ($–CH_2$)
2853	C–H	Symmetrical stretching	Aliphatic ($–CH_2$)
1743	C=O	Stretching	ν(C=O) ester
1654	C=C	Deformation	δ(C=C) acyl
1462	C–H	Scissoring	Aliphatic ($–CH_2$)
1377	C–H	Symmetrical deformation	Aliphatic (CH_3)
1238	C–H	Out-of-plane bending	Aliphatic ($–CH_2$)
1162	C–O	Stretching	ν(C–O) ester
1025	C–O–C	Stretching	ν(C–O–C) ester
966	C–H	Out-of-plane bending	*trans* (–CH=CH–)

Meanwhile, the FTIR spectra of animals, palm as well as the fish pellets oil look very similar due to the fact that the main component composed of these oils are solely triglycerides. However, due to the fingerprint technique on the region of 650 – 1500 cm^{-1}, meaning that there is no two compounds

or samples having the same spectra in terms of amount and intensity of peaks, FTIR spectroscopy can be used to extract the differences among these oils. Upon closer scrutiny, the minor differences (peak heights) between all the animals' and palm oils at 1117 and 1097 cm^{-1} corresponding to C-H bending vibration and C-H deformation vibrations of fatty acids, are observed as reported beforehand [21]. Furthermore, these frequencies, where the FTIR spectra variations were observed, are used as the basis for choosing the spectral regions in quantification and classification of animal's and plant's oil.

Noteworthy, while the FTIR-ATR technique offers promising advantages for *halal* verification, it is essential to acknowledge and address its potential challenges and limitations to ensure its effective and reliable implementation. One of the primary challenges associated with FTIR-ATR spectroscopy is the complexity of food matrices, which can lead to overlapping spectral bands and interfering signals. Food products often consist of intricate mixtures of various components, such as proteins, carbohydrates, lipids and additives, making it difficult to accurately identify and quantify specific non-*halal* substances. This issue is particularly prominent in processed or multi-ingredient food products, where the spectral signatures of individual components may be obscured or masked [22]. Moreover, the reliability of FTIR-ATR for *halal* verification heavily depends on the availability of comprehensive spectral databases and robust chemometric models. Building and maintaining these databases and models requires extensive research efforts, involving the collection and analysis of a wide range of *halal* and non-*halal* reference materials. Inadequate or incomplete databases may lead to misidentification or inaccurate quantification of *halal* and non-*halal* components. Owing that the FTIR-ATR is a relatively simple and non-destructive technique, proper sample preparation and handling are crucial for accurate analysis. Factors such as sample homogeneity, particle size and surface contact can influence the quality of the obtained spectra. Inconsistencies in sample preparation or handling procedures may introduce variability and affect the reliability of the FTIR-ATR spectrum results. Hence, to address these challenges and limitations, ongoing research efforts are necessary to improve sample preparation methods, develop more robust chemometric models and expand spectral databases to encompass a wider range of *halal* and non-*halal* reference materials [23].

Although there are several environmental and handling conditions that can potentially affect the accuracy of results obtained from FTIR-ATR spectroscopy when used for *halal* verification purposes. Addressing these factors is crucial to ensure reliable and reproducible analytical outcomes. The FTIR-ATR spectroscopy is sensitive to changes in temperature and humidity, which can influence the molecular vibrational modes and lead to spectral variations. Fluctuations in these environmental conditions during sample analysis can introduce inconsistencies in the acquired spectra, potentially compromising the accuracy of component identification and quantification. However, to mitigate these effects, it is essential to maintain a controlled and consistent temperature and humidity environment within the FTIR-ATR instrument. This can be achieved through appropriate climate control systems or by conducting analyses in temperature and humidity-controlled rooms or enclosures. Next, the accuracy of FTIR-ATR results is heavily dependent on proper sample preparation and handling techniques. Factors such as sample homogeneity, particle size distribution and surface contact with the ATR crystal can significantly impact the quality of the obtained spectra. Insufficient sample homogenization or the presence of large particles can lead to inconsistent and unrepresentative spectral data, potentially masking or misrepresenting the presence of non-*halal* components. Improper contact between the sample and the ATR crystal can also result in poor signal-to-noise ratios and decreased spectral quality. Hence, to address these challenges, standardized sample preparation protocols should be established, including appropriate grinding, mixing and compression techniques to ensure homogeneity and optimal sample-crystal contact. Additionally, careful handling and cleaning procedures for the ATR crystal are crucial to prevent cross-

contamination between samples and maintain consistent analytical conditions. By addressing these environmental and handling conditions, *halal* certification bodies and regulatory authorities can enhance the accuracy and reproducibility of FTIR-ATR analyses for *halal* verification purposes [24].

Table 2
Difference of functional groups present in the beef, chicken, pork and palm oils

Wavenumber (cm^{-1})					Functional Group	Vibration Model	Intensity
Beef Oil	Chicken Oil	Pork Oil	Palm Oil	Reference [25]			
3003	3006	3006	3005	3000	C=C–H (*cis*–	Stretching	Weak
2953	2951	2952	2952	2960	C–H (CH_3)	Asymmetric Stretching	Medium
2917	2920	2920	2920	2930	C–H (CH_2)	Asymmetric Stretching	Strong
2849	2851	2851	2850	2850	C–H (CH_2)	Symmetrical Stretching	Strong
1743	1744	1743	1744	1750	C=O (esters)	Stretching	Strong
-	1653	1652	-	1650	C=C (*cis*-)	Stretching	Weak
1465	1457	1464	1456	1470	C–H (CH_2, CH_3)	Cut-Out-Bend	Strong
1377	1377	1377	1377	1380	C–H (CH_3)	Symmetrical Stretching	Medium
1235	1236	1235	1235	1240	C–O (in esters)	Stretching	Medium
1160	1159	1159	1159	1160	C–O (in esters)	Stretching	Strong
1096	1096	1095	1097	1100	C–O (in esters)	Stretching	Medium
965	964	967	-	1000	C=C-H (*trans*-)	Out the Field-Bend	Medium
721	722	721	721	720	– $(CH_2)_n$–	Wobble-Bend	Strong

Table 3
Difference of functional groups in the imported fish pallets samples

Wavenumber (cm^{-1})				Functional Group	Vibration Model	Intensity
Sample #1	Sample #2	Sample #3	Reference [25]			
3008	3007	3008	3000	C=C–H (*cis*–)	Stretching	Weak
2954	-	-	2960	C–H (CH_3)	Asymmetric Stretching	Medium
2921	2920	2921	2930	C–H (CH_2)	Asymmetric Stretching	Strong
2852	2851	2851	2850	C–H (CH_2)	Symmetrical Stretching	Strong
1743	1742	1742	1750	C=O (esters)	Stretching	Strong
-	-	-	1650	C=C (*cis*–)	Stretching	Weak
1462	1464	1463	1470	C–H (CH_2, CH_3)	Cut-Out-Bend	Strong
1377	1376	1377	1380	C–H (CH_3)	Symmetrical Stretching	Medium
-	1236	1237	1240	C–O (in esters)	Stretching	Medium
1164	1160	1161	1160	C–O (in esters)	Stretching	Strong
-	1116	1116	1100	C–O (in esters)	Stretching	Medium
1060	-	1097	1000	C=C-H (*trans*–)	Out the Field-Bend	Medium
721	721	721	720	– $(CH_2)_n$–	Wobble-Bend	Strong

3.2 Halal Verification of Imported Fish Pellets through the Principal Component Analysis

Although there is a lack in this research that have mentioned any dataset transformation, thus, it is recommended that all the variables must be transformed using the standard deviation (n-1) approach. The dataset transformation simultaneously corrects the linearity issue as well and so the linearity test is on a case-by-case basis. The last step before multivariate data analysis is performing the assumption testing, which involves normalization. The normalization of the dataset was conducted by performing the Shapiro-Wilk test at α = 0.01, which allows only 1% chance of false positive in this research as to designed for verification analysis. Therefore, it should be very effective in reducing errors [26].

The PC with an eigenvalue of less than a unit account for less variance than did the original variable (which had a variance of 1.000 unit) and so are of little use and usually such a component should be eliminated so that fewer components are dealt with. The PCA and scree plots are powerful tools in multivariate data analysis that can facilitate the generalization of different data for *halal* verification of various meat products. These techniques allow researchers to extract relevant information from complex and high-dimensional datasets, such as those obtained from analytical techniques like FTIR-ATR spectroscopy. In the context of *halal* verification of meat products, PCA can be applied to the FTIR-ATR spectral data obtained from different types of meat samples (e.g., chicken, beef, pork). The principal components capture the most significant sources of variation within the dataset, which can be attributed to factors such as the presence or absence of non-*halal* components, compositional differences or processing methods. In addition to the value in eigenvalue, the scree plot which consists of a simple line segment that shows the fraction of total variance in data also able to visualize in determining an appropriate number of PC. Thus, from the PCA of 1027 dataset in FTIR fingerprint region, 1500 – 650 cm^{-1}, the first four PC of the FTIR region 1000 – 650 cm^{-1} whereas the first five on the region of 1500 – 1001 cm^{-1} region were observed with the eigenvalue more than 1.000 unit (Tables 4 and 5) as the scree plot line (Figures 2 and 3) after the fifth and sixth PC was almost flat indicating that the corresponding PC were accounted for lesser amounts of the cumulative variability, respectively.

Table 4
The eigenvalue of the PC and their contributions to the variability of all extracted samples using FTIR-ATR of 1027 dataset at the region of 1000 – 650 cm^{-1} regions (where F = PC = principal component)

	F1	F2	F3	F4	F5	F6
Eigenvalue	470.459	244.465	10.384	2.063	0.509	0.121
Variability (%)	64.623	33.580	1.426	0.283	0.070	0.017
Cumulative %	64.623	98.204	99.630	99.913	99.983	100.00

Table 5
The eigenvalue of the PC and their contributions to the variability of all extracted samples using FTIR-ATR of 1027 dataset at the region of 1500 – 1001 cm^{-1} region

	F1	F2	F3	F4	F5	F6
Eigenvalue	786.316	186.390	44.666	15.715	3.213	0.700
Variability (%)	75.826	17.974	4.307	1.515	0.310	0.067
Cumulative %	75.826	93.800	98.107	99.623	99.933	100.00

In the meanwhile, the scree plots are complementary tools used in conjunction with PCA to determine the appropriate number of principal components to retain for further analysis or model development. These plots illustrate the percentage of variance explained by each principal component, enabling researchers to identify the point at which the addition of subsequent components contributes negligible explanatory power. In the context of *halal* verification, the scree plot can assist in selecting the optimal number of principal components that capture the majority of the relevant variation in the FTIR-ATR spectral data while minimizing the influence of noise or redundant information. By retaining only the significant principal components, researchers can effectively reduce the dimensionality of the dataset, facilitate interpretation and enhance the robustness of subsequent multivariate models or classification techniques. The combination of principal component plots and scree plots allows researchers to generalize the spectral data for different meat products, visualizing the inherent patterns and identifying the key sources of variation

that distinguish *halal* from non-*halal* samples. This generalization is crucial for developing robust and reliable classification models or identifying potential non-*halal* components based on their unique spectral signatures.

Based on the eigenvalues of each PC and the appearance of the scree plot, it has been confirmed that the quantitative traits with a higher factor loading on F1, F2, F3 and F4 (for 1000 – 650 cm^{-1} region) and F1, F2, F3, F4 & F5 (for 1500 – 1001 cm^{-1} region) contributed the most variability observed in the corresponding six quantitative traits where a great emphasis had been given for those traits having higher loading on the first four and five PC.

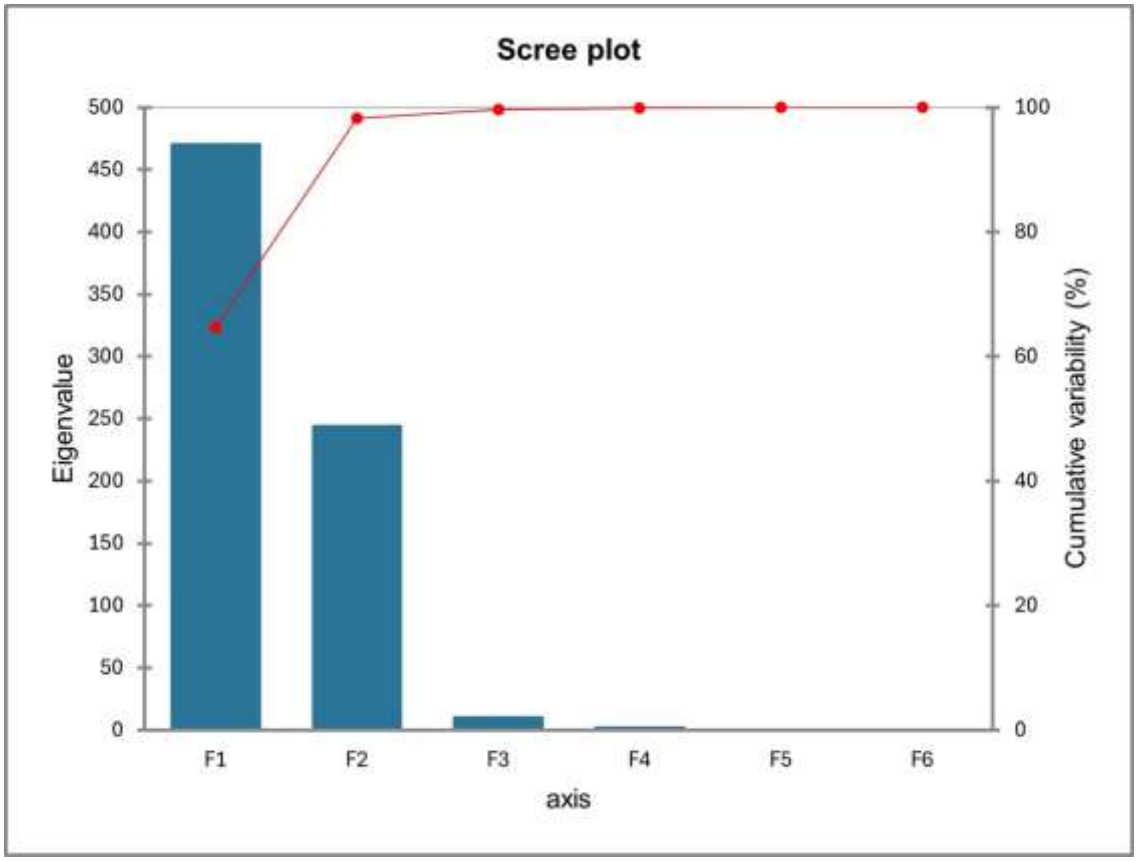

Fig. 2. The scree plot of eigenvalues and variance explained by the PCA of extracted animal's, palm and fish pellets oils of 1027 dataset at the FTIR region of 1000 – 650 cm^{-1}

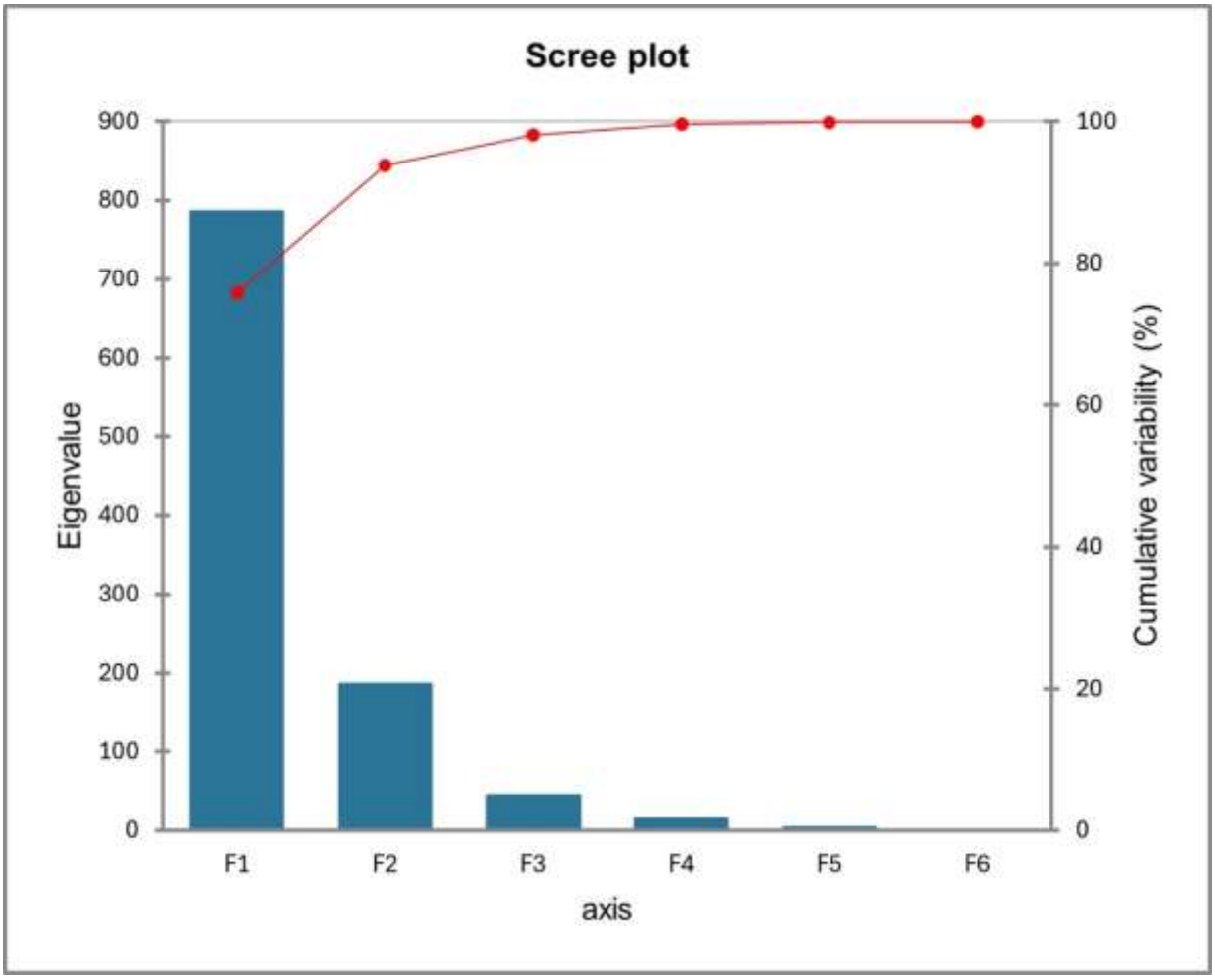

Fig. 3. The scree plot of eigenvalues and variance explained by the PCA of extracted animal's, palm and fish pellets oils of 1027 dataset at the FTIR region of 1500 – 1001 cm^{-1}

The reason of conducting PCA is to simplifies the complexity in high-dimensional data as it is showing the trends and patterns between the animal's oils and the imported fish pellets in terms of ingredients manufacture used throughout process. Moreover, the PCA projects the original data in reduced dimensions defined by the PCs. This technique is useful when there is a correlation between data [27].

Table 6
The KMO test produced a KMO score for 1037 dataset from all extracted animals', palm and imported fish pellets oils of the FTIR fingerprint's region, 1500 – 650 cm^{-1}

Kaiser-Mayer Measure of Sampling Adequacy :
KMO (1000 – 650 cm^{-1} region) = 0.565
KMO (1500 – 1001 cm^{-1} region) = 0.559

All the beef, chicken, pork and palm oils as well as the imported fish pellets was classified using chemometric of PCA. The wavenumber of all the sample were separate at the fingerprint's regions of 1000 – 650 cm^{-1} and 1500 – 1001 cm^{-1}, respectively. These regions were chosen due to its capability to provide the good separation among all the evaluated samples.

The Figures 4 and 5 demonstrates the score plot of PCA of all the beef, chicken, pork and palm oils and the imported fish pellet, representing the projection of samples defined by the first PC1 and

the second PC2 at the fingerprint regions of 1000 – 650 cm^{-1} and 1500 – 1001 cm^{-1}, respectively. Moreover, Figure 4 display factor loading (FL) of 64.42 % and 33.58% along with observations value of 98.20% while Figure 5 display FL of 75.83% and 17.97% with observation value of 93.80% for whole dataset, respectively. This observation value indicated that all the reading from FTIR spectrum for all extracted animals', palm and imported fish pellets oils at the fingerprint's region of 1500 – 650 cm^{-1} could explain the dataset very well as the CV value was greater than 90% [28].

Utilizing these projections of PCA, it clearly seen that the imported fish pellets of sample #1, #2 and #3 does not contain or adulterated with pork oil (lard) as all the imported fish pellets samples are separated far from the sources through both 1000 – 650 cm^{-1} and 1500 – 1001 cm^{-1}, respectively. Nevertheless, as thorough inspections are conducted, it is also possible to speculate that the imported fish pellets contain animal by-products from terrestrial sources, as numerous researchers have recently found [29]. It is mentioned that the *halal* verification procedure cannot rely only on *Shariah* knowledge; it also needs to take into account the competence of other associated scientific domains including chemistry, food science and technology and veterinary science [30]. In addition, *halal* verification nowadays calls for the use of the most recent, extremely sophisticated technology and analytical instrumentation rather than relying solely on physical examination and documentation. Due to new problems that are uncommon in traditional *fiqh*, the aforementioned claim of adulteration of *haram* or *shubhah* substances in food goods has been widely dispersed and is difficult to detect with the naked eye [31].

The integration of FTIR-ATR spectroscopy with multivariate data analysis techniques, notably PCA, has garnered significant attention in the field of *halal* verification. PCA is a widely employed chemometric method for extracting relevant information from the high-dimensional and complex spectral data obtained from FTIR-ATR measurements [32]. However, the robustness of this approach is contingent upon several critical factors that must be carefully evaluated and addressed. The reliability of PCA models for *halal* verification is heavily influenced by the representativeness and quality of the spectral dataset used for model development. It is crucial to ensure that the dataset encompasses a diverse range of *halal* and non-*halal* reference materials, encompassing various food matrices, concentrations and potential interferences. Furthermore, inadequate representation of the inherent variability in the data can lead to overfitting or poor generalizability of the PCA models, compromising their ability to accurately classify new or unseen samples. Furthermore, rigorous spectral preprocessing steps, such as baseline correction, normalization and noise reduction, are essential to enhance the signal quality and mitigate potential artifacts or distortions in the FTIR-ATR spectra. One of the advantages of PCA is its ability to provide insights into the underlying chemical signatures responsible for the observed spectral variations. By examining the loadings and scores plots generated from the PCA analysis, researchers can identify the spectral regions and corresponding molecular vibrations that contribute significantly to the classification of *halal* and non-*halal* samples. However, the interpretability and chemical relevance of these findings should be carefully evaluated and validated against established chemical knowledge and reference data. This process not only enhances the understanding of the analytical process but also facilitates the development of more robust and interpretable PCA models for *halal* verification. While FTIR-ATR and PCA offer a powerful combination for *halal* verification, it is essential to validate and corroborate the findings with complementary analytical techniques. This approach can mitigate potential limitations or biases inherent to a single analytical method and provide a more comprehensive and reliable assessment of the *halal* status of food products. Techniques such as chromatographic methods, mass spectrometry or other spectroscopic techniques can be employed to cross-validate the results obtained from FTIR-ATR and PCA, enhancing the overall confidence in the *halal* verification process [33].

The interpretation of FTIR-ATR spectra plays a crucial role in understanding the chemical basis for distinguishing *halal* from non-*halal* products. By leveraging the unique molecular vibrational signatures captured in the infrared spectra, researchers can gain insights into the compositional differences and identify the presence of potential non-*halal* components. However, the complexity of food matrices and the overlapping spectral features often necessitate the application of multivariate analysis techniques to extract meaningful information from the high-dimensional FTIR-ATR data. As the FTIR-ATR spectra provide a detailed fingerprint of the molecular composition of a sample, with each peak or band corresponding to specific vibrational modes of functional groups or chemical bonds. In the context of *halal* verification, the identification and interpretation of these characteristic bands can provide valuable insights into the presence or absence of non-*halal* substances. For instance, the presence of distinctive bands associated with porcine-derived materials, such as specific protein or lipid signatures, can serve as indicators of potential non-*halal* components. Similarly, the characteristic bands of non-*halal* animal fats or gelatine derived from non-*halal* sources can be identified and compared against reference spectra to assess the *halal* status of a product [34].

The multivariate analysis techniques, like PCA, are powerful tools for extracting relevant information from the high-dimensional FTIR-ATR spectral data. PCA transforms the original spectral variables into a new set of uncorrelated variables called PCs, which capture the maximum variation present in the data. By projecting the spectra onto these PCs, researchers can visualize the inherent patterns and clusters within the data, facilitating the discrimination between *halal* and non-*halal* samples. The scores plots generated from PCA can reveal distinct groupings or separations based on the presence or absence of non-*halal* components, even when the spectral differences are subtle or obscured by other factors. Furthermore, the loadings plot in PCA provide insights into the specific spectral regions or wavenumbers that contribute most significantly to the observed patterns and separations. By correlating these loadings with known spectral signatures and chemical knowledge, researchers can identify the molecular vibrations and functional groups responsible for distinguishing *halal* from non-*halal* products [35].

Because of the limited technology available at the time, Muslim scholars used their own senses (observation) to rule over *hukms* (or acts) without the need for empirical or scientific proof. Fundamentally, their perspectives are no longer appropriate for dealing with new, complex situations like the ones that are occurring at the present time [36]. Owing to technological advancements, we can now use FTIR-ATR instruments to detect porcine by-products in a lot of foods and pharmaceutical products as reported throughout researchers within the Muslim countries. Further, some concerns that are happening in our technological age are modern and have never been covered by traditional Muslim scholars, such as the controversy surrounding meat that is grown in laboratories [37].

Therefore, in order to address this issue, scientific research is frequently required in order to support the lawmakers (*mujtahid*) in defining Islamic ruling through the collective application of ijtihad methodology. In our situation, collective *ijtihad* is very applicable and useful since certain problems require effective answers, such as in other sectors that are too complex for a single person to specialize in. Therefore, in making Islamic ruling, Muslim scholars should cooperate with other scholars from other fields of study to determine a decision from *ijtihad* with efficacy [38].

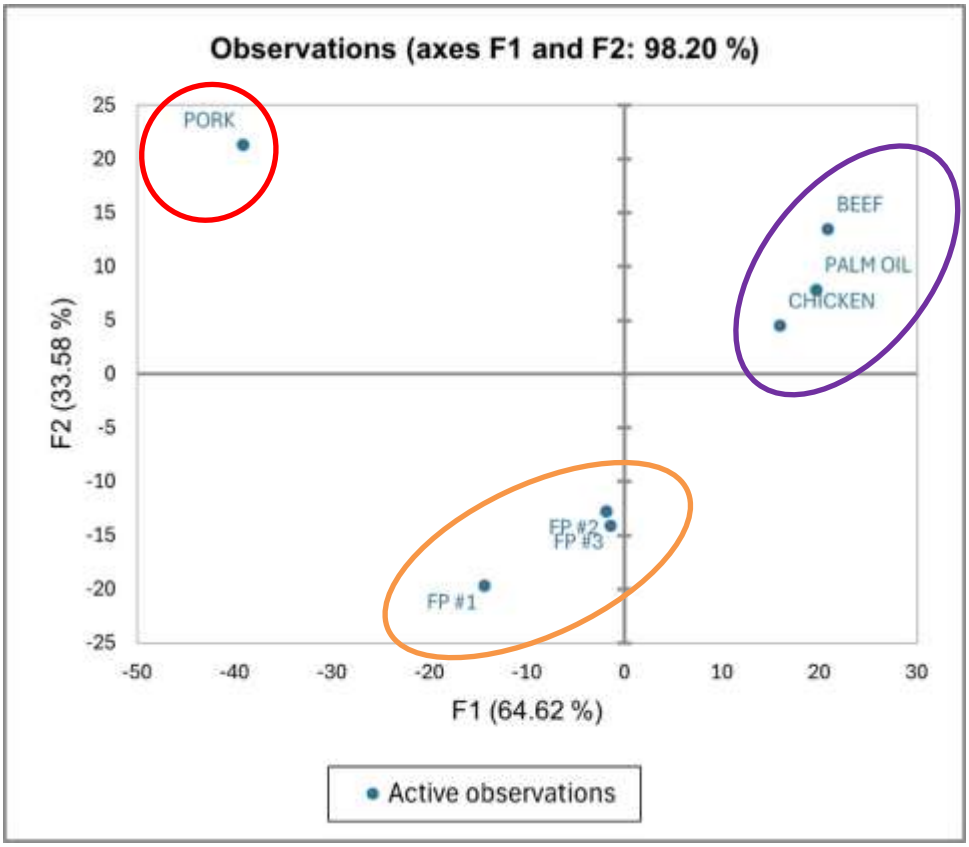

Fig. 4. The PCA of extracted animal's, palm and fish pellets oil dataset with observation value of 98.20% at the FTIR fingerprint region of 1000 – 650 cm^{-1}

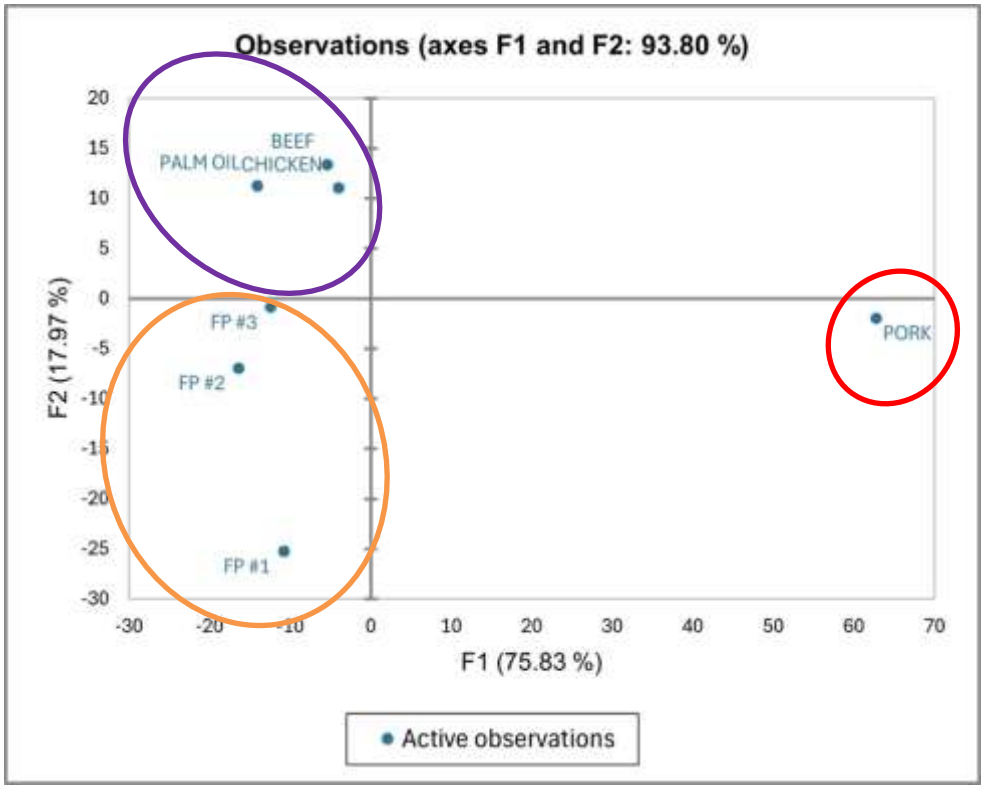

Fig. 5. The PCA of extracted animal's and fish pellets oil dataset with observation value of 93.80% at the FTIR fingerprint region of 1500 – 1001 cm^{-1}

4. Conclusions

In conclusion, the FTIR-ATR spectroscopy combined with PCA offers a robust method for identifying and distinguishing imported fish pellets based on the source of oil used in manufacturer's formulation. This approach holds significant promise for *halal* verification processes, aligning with the strict dietary guidelines mandated by Islamic principles. By analysing the FTIR spectral data of the extracted fats within the specific fingerprint regions which are at 1000 – 650 cm^{-1} and 1500 – 1001 cm^{-1}, the PCA results provide a means to not only differentiate between various sources but also detect any potential adulteration or unauthorized additive in the samples. This comprehensive analytical strategy not only ensures adherence toward the *halal* standards but also enhances food safety and authenticity protocols in the fish pellet industry, contributing to consumer's trust and confidence in the market. However, additional of other spectroscopy method can be added to further verification the content of all fish pellets samples such as Raman spectroscopy, NMR spectroscopy and the UV-visible spectroscopy which based on the interaction of electromagnetic radiation can be a promising tool for screening and identification of porcine by-product and non-*halal* meats through oil as a medium in all food-based and pharmaceutical products.

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