Malaysian Journal of Chemistry, 2024, Vol. 26(6), 184-193

The FTIR-ATR Spectroscopy and Multivariate Data Analysis (MVDA) for *Halal* Authentication of Animal Fatty Acids

Muhammad Zulhelmi Nazri[1], Siti Nor Azlina Abd Rashid[1], Salimah Ab Malik[1], Hajar Aminah A. Karim[1], Muhamad Shirwan Abdullah Sani[2] and Dayang Norulfairuz Abang Zaidel[1,3]*

[1]Innovation Centre in Agritechnology for Advanced Bioprocessing (ICA), Universiti Teknologi Malaysia (Pagoh Campus), Eduhub Tinggi Pagoh, 84600 Pagoh, Muar, Johor, Malaysia

[2]International Institute for Halal Research and Training (INHART), Level 3, KICT Building, International Islamic University Malaysia, Jalan Gombak 53100 Kuala Lumpur, Malaysia

[3]Department of Chemical and Environmental Engineering, Malaysia-Japan International Institute of Technology (MJIIT), Universiti Teknologi Malaysia 54100 Kuala Lumpur, Malaysia

*Corresponding author (e-mail: dnorulfairuz@utm.my)

The authentication of *halal* products is crucial for adherents of Islam, as consuming non-permissible substances contradicts religious mandates. The recent widespread adulteration of food and pharmaceutical products with porcine-derived ingredients has necessitated the development of robust analytical methods for *halal* verification. This study presents an approach for rapid *halal* authentication using Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR), combined with multivariate data analysis (MVDA). Animal fats, i.e., beef, chicken, pork (lard), and goat, and a plant-based oil, i.e., palm oil, were extracted via Soxhlet apparatus utilising petroleum ether as solvent. The FTIR-ATR spectra were acquired in the mid-infrared region (4000 – 650 cm^{-1}), encompassing both fingerprint and functional group regions. Principal component analysis (PCA) was employed to identify unique spectral patterns and develop classification models for *halal* authentication. The combination of FTIR-ATR and MVDA techniques enables the identification of characteristic spectral features and developing classification models for *halal* authentication. The PCA results revealed distinct clustering of samples based on their origin, with total variance range of 74.75 – 98.79%, explained by the first two principal components based on all wavenumbers in the 4000 – 650 cm^{-1} FTIR spectra. This FTIR-ATR coupled with the MVDA approach offers a rapid, non-destructive, and cost-effective method for *halal* authentication. The approach's high sensitivity and specificity make it a promising tool for regulatory bodies and food manufacturers to ensure compliance with *halal* standards.

Keywords: Animal oils; FTIR spectroscopy; multivariate data analysis; principal component analysis; *Halal* authentication

Received: August 2024; Accepted: November 2024

Halal is an Arabic term that describes foods, in accordance with Islamic law, that Muslims are permitted to consume [1]. Moreover, unless extremely exceptional circumstances apply, members of Muslim society are not permitted to consume any products containing non-*halal* ingredients [2]. In the meanwhile, *halal* products-which include foods and pharmaceuticals, are any goods that contain components that are allowed by Islamic law and meet specific requirements: (a) do not include any animal products or parts that are not permissible by Islamic law to be consumed, nor do they contain any animal parts that are not slaughtered in accordance with Islamic law; (b) do not include *najs* (for example, animals such as amphibians, pig and its derivatives, blood, and carrions); (c) safe for human consumption, as such, not dangerous, not intoxicating or not harmful to health when used in accordance with recommended dosage; (d) not prepared, processed or manufactured using equipment contaminated with *najs*; (e) do not contain any human parts or its derivatives that are not permitted by Islamic law; and (f) during its preparation, processing, handling, packaging, storage and distribution, the *halal* pharmaceutical products are physically separated from any other pharmaceutical products that do not meet the requirements stated in items (a), (b), (c), (d), and (e), or any other items that have been decreed as non-*halal* and *najs* through Islamic law [3].

As mentioned above, all *halal* products must be free from non-*halal* components, which are pig and all its derivatives (such as pork, lard and porcine gelatine), carrion, blood (flowing or congealed), animals slaughtered not according to Islamic law, animals that were killed accidentally or on purpose through means such as strangling or beating, intoxicants including drugs and alcohol [3], carnivorous animals, predatory birds, and certain land animals [4]. Among all, pig derivatives and alcohols are typically found in *halal* food products, thus researchers are continuously

researching in *halal*-related issues, including developing instrumental analytical methods for detecting non-*halal* components intended for *halal* authentication [5].

Edible fats and oils are considered essential components of food products. Besides that, in the recent year, animal fats and vegetable oils have been considered as economic sources for food and oleochemical and pharmaceutical industries [6]. Nutritionists recommend that people consume vegetable oils as a source of essential fatty acids such as oleic, linoleic and α-linolenic acids and fat-soluble vitamins, which are A, D, E and K, needed in human metabolism [7]. Noteworthy, the quality of food products containing fats and oils depends on the quality of the fats and oils, including the authenticity, purity, and some intrinsic quality parameters [8]. However, adulteration of fats and oils has been widespread in the food industry, involving the replacement of higher value products with lower grade, cheaper, and more readily substitutes. The authenticity of fats and oils has been extensively investigated because they can easily be adulterated due to economic purposes [9]. Mixing of animal fats with vegetable sources is a cause of concern for certain groups of consumers due to religious obligations and health complications [10].

Infrared spectroscopy has drawn interest in the analytical community for use in the quantitative quantification of fats and oils. Meanwhile, because infrared is a vibrational type of spectroscopy and offers quick evaluation while being cost-effective, it is an excellent analytical approach for analysing food and pharmaceutical products. The most common technique for food analysis in infrared spectroscopy is in the mid-infrared region (4000 - 400 cm^{-1}) and near-infrared region (14000 – 400 cm^{-1}) [11]. The fundamental concept underlying infrared spectroscopy is that samples interact with electromagnetic radiation in the infrared range, which causes vibrational transitions in the molecules in the samples. Samples can be placed directly on an ATR crystal for measurement, requiring less analysis time and less solvent application. Another advantage of adopting the attenuated total reflectance (ATR) spectroscopic technique for FTIR is the simplicity of in-sample preparation [12]. This simplification reduces the time and effort required for analysis and minimises the risk of sample contamination or alteration, thereby enhancing the reliability and reproducibility of spectral data. The use of FTIR spectroscopy in combination with chemometrics for the analysis of non-*halal* components, including pig derivatives and several non-*halal* meats such as wild boar, dog, and rat meats, is widely reported due to its functionality. This is reported due to its advantages, particularly in the fingerprint analytical technique of the FTIR instrument [13]. Distinguishing between animal fats is critical for *halal* authentication, as fats from non-*halal* sources like pork or improperly slaughtered animals are prohibited for Muslim consumption. Ensuring the source of fat is compliant builds consumer trust and meets religious requirements. Using FTIR and PCA is significant in this process, as FTIR detects unique molecular "fingerprints" of fats, while PCA simplifies complex data, enhancing the accuracy of fat identification. Together, these techniques provide a fast, reliable, and non-destructive method for ensuring *halal* integrity in products, preventing contamination with non-*halal* substances, as has been previously described [14].

The FTIR/MVDA approach has proven particularly effective in detecting porcine derivatives and other non-*halal* meats, including wild boar, dog, snake, and rat. The widespread adoption of this approach can be attributed to the unique fingerprinting capabilities of FTIR spectroscopy, which allows for the identification of specific molecular structures and functional groups characteristic of these non-*halal* components. The synergy between FTIR spectroscopy and multivariate data analysis approach, such as PCA and cluster analysis, is compelling, and has been demonstrated to be particularly powerful in detecting and quantifying of lard adulteration in oils [13]. PCA reduces complex spectral data into manageable principal components, enabling the visualisation of sample patterns and differences among samples. Cluster analysis further enhances this approach by grouping similar spectra, thereby aiding in the identifying of adulterated samples. This combination of techniques offers a robust, rapid, and non-destructive method for ensuring food authenticity and compliance with *halal* standards, addressing a critical need in the food industry and regulatory sectors [14].

EXPERIMENTAL

Experimental Design

The current study entailed extracted animal oils of chicken, beef, pork, and goat, as well as standard palm oil by Fourier transform infrared attenuated total reflectance (FTIR-ATR) spectroscopy. The dataset was subjected to dataset pre-processing and principal component analysis (PCA). **Figure 1** shows the experimental design of the current study.

Materials

Adipose tissues of chicken, pork, goat, and beef were obtained from local supermarkets. The analytical solvent used for fat extraction was petroleum ether (boiling range: 60 – 80°C, R&M Chemicals, UK). Palm oil (Sigma-Aldrich, USA) was used as standard with no further treatment. $MgSO_4$ was obtained from R&M Chemicals, UK. All chemicals used were of analytical grade and used without further purification.

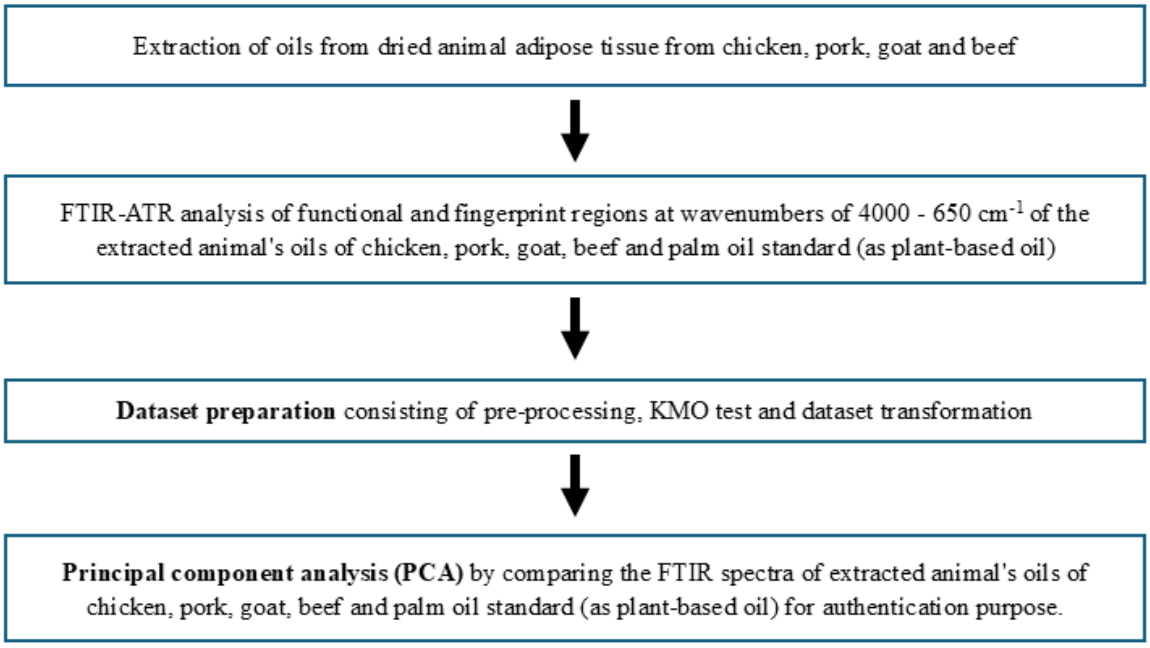

Figure 1. Experimental design of incorporation of principal component analysis with Fourier transform infrared spectroscopy with attenuated total reflectance for *halal* authentication of animal fatty acids.

Treatment of Animal Adipose Tissues

All the animal adipose tissues (chicken, pork, goat, and beef) were cut into 1 cm × 1 cm size using a commercial cutter and freeze-dried at 0.32 bar for 24 h. The freeze-dried tissues were stored in a freezer (Sharp, Japan) at -20°C until further analysis.

Fat Extraction from Animal Adipose Tissues

The fats from chicken, pork, goat, and beef were extracted according to the established methodology with minor modifications [15]. About 20 g of dried sample was weighed and grinded into a fine powder using a commercial blender (Wiring, USA), before being put into a cellulose 30 × 100 mm extraction thimble. Then, the top of the thimble was covered with cotton wool, as to prevent the sample from escaping before being inserted into the Soxhlet apparatus. The extraction process was carried out for 6 h using petroleum ether as the solvent. The obtained extracts were mixed with a spoonful of $MgSO_4$ to remove moisture, filtered through a 125 mm ashless filter paper (Whatman, UK), which later evaporated using a rotary evaporator. The dried extracted fats and oils were stored in glass vials.

FTIR Measurement

The Nicolet iS5 spectrophotometer (Thermo Scientific, USA), equipped with a diamond cell of attenuated total reflectance (ATR) accessory, was used in the measurements. All measurements were performed in a dry atmosphere at room temperature (25 ± 0.5 °C) on a single drop of the extracted animal and palm oils. All spectra were recorded within a range of 4000 – 600 cm^{-1} at 4 cm^{-1} resolution and 32 scans. Prior to sample measurement, a background spectrum was recorded. Then, a spectrum was obtained for each measurement. The sample's spectrum was subtracted against the background spectrum, and the result was presented in transmittance units. Three spectral replicates were obtained from three independent samples.

Data Pre-Processing

The spectra were compiled into comma-separated values (CVS) format file and exported to the XLSTAT software (2024 version, France) [16]. The transmittance of the spectrum was separated into different wavenumber ranges, i.e., 4000 – 3501 cm^{-1}, 3500 – 3001 cm^{-1}, 3000 – 2501 cm^{-1}, 2500 – 2001 cm^{-1}, 2000 – 1501 cm^{-1}, 1500 – 1001 cm^{-1}, and 1000 – 650 cm^{-1}. The most significant wavenumbers were selected from PLS-DA, and PCA was performed to find the apportionment of wavenumbers of the animals' fatty acids [17].

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 [18].

Dataset Transformation

To ensure that the dataset followed a normal distribution before the PCA, the dataset normality was tested using Shapiro-Wilk test at $\alpha = 0.01$. The dataset was transformed using standard deviation (n-1) methods [19].

Analysis using Principal Component Analysis (PCA)

The whole FTIR spectra was extracted its transmittance value to obtain the dataset for PCA. The FTIR spectra at all the wavenumbers in the range of 4000 – 650 cm^{-1}. Analysis of PCA was performed using the XLSTAT software [16], and the data were scaled using Pareto scaling technique prior to PCA analysis to maximise the variation. Pareto scaling helps by reducing the dominance of high-variance variables by ensuring that principal components (PC) better represent the true underlying data structure rather than being skewed toward variables with large ranges. After Pareto scaling, the variables used for the PCA model were more normally distributed, shown by its Gaussian curve. The number of PCs was optimised to obtain optimum differentiation among samples. The differentiation result of samples was observed using PCA score plot. Moreover, the 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.

RESULTS AND DISCUSSION

FTIR-ATR Spectra Analysis

The extracted oils from dried beef and chicken had a similar physical appearance, which was yellow, whereas the palm oil was in different colours. On top of that, all the fatty acids demonstrated similar FTIR spectra and were obviously difficult to distinguished among them, as depicted in **Figure 2** (overlay) and **Figure 3** (stacked). Furthermore, all the oil samples of the animals and palm oil presented triacylglycerols, triglycerides, and fatty acids as the main compositions of fats in the oils, as reported previously [20]. All the spectra showed the typical characteristics of absorption bands of animal fatty acids. The resulting FTIR vibrations from all the oil samples were mainly from those compounds. The stretching vibrations of -CH, CH_2 and CH_3 from aromatic and alkene could be observed at a peak of 3000 cm^{-1}, whereas the stretching vibrations of –CH, CH_2 and CH_3 from aliphatic alkane were found at peaks of ~2900 – 2800 cm^{-1}. It was observed that all the oil samples, regardless of animal or plant origin, had a sharp and intense peak at the carbonyl (C=O) region of ~1700 cm^{-1}. Next, the absorption band at ~1400 cm^{-1} was correlated to the stretching vibration of C=C. On the other hand, absorption bands at 1100 – 1000 cm^{-1} arose from the vibration of C–O stretching. In addition, vibrations at 1200 – 700 cm^{-1} were associated with bending vibrations of –CH, CH_2 and CH_3 fatty acid aliphatic backbone [21].

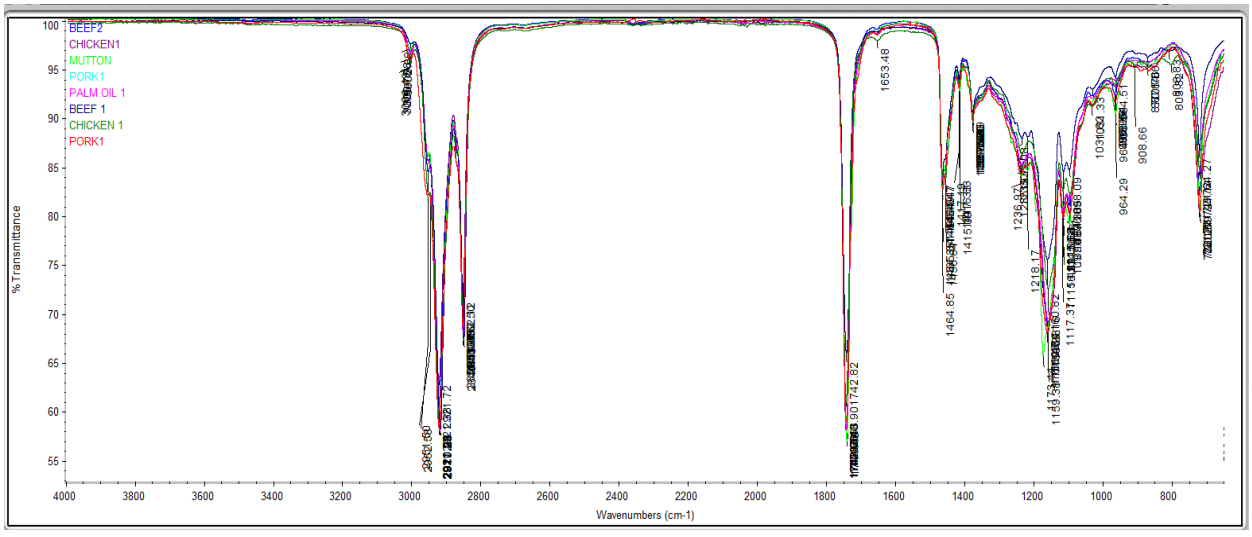

Figure 2. Full overlay of FTIR spectra of animal oils (beef, chicken, mutton, pork) and palm oil of wavenumber range of 4000 – 600 cm^{-1}.

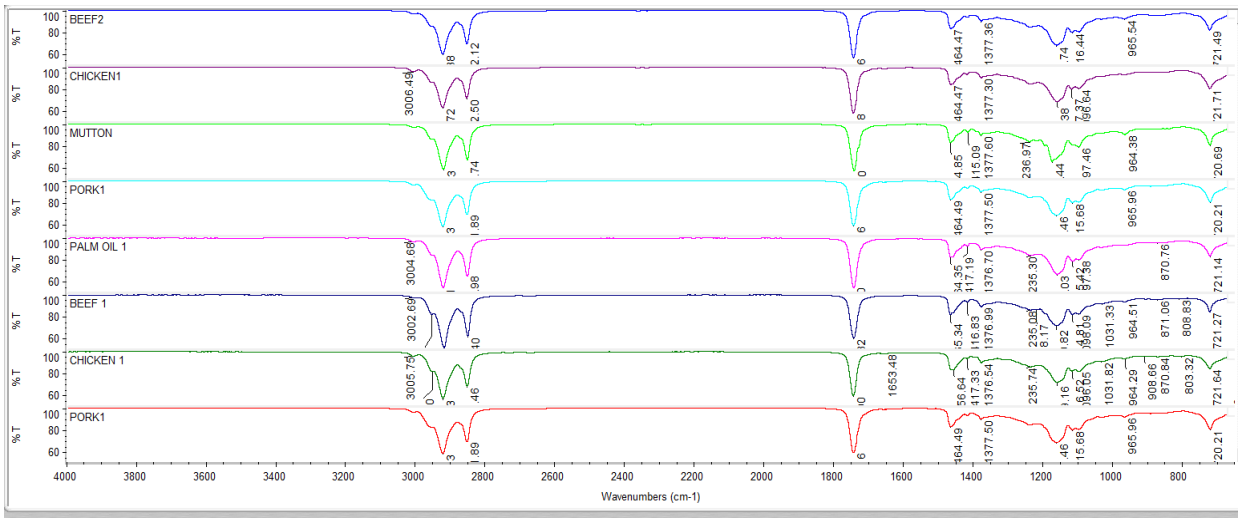

Figure 3. Stacked FTIR spectra of animal oils (beef, chicken, mutton, pork) and palm oil of wavenumber range of 4000 – 600 cm^{-1}; noticing that there are undistinguishable spectrum peaks among all oil samples.

Table 1. KMO test scores of 728 datasets.

The Kaiser-Mayer Measure of Sampling Adequacy
KMO (for the 1000 – 650 cm^{-1} region) : 0.757
KMO (for the 1500 – 1001 cm^{-1} region) : 0.641
KMO (for the 2000 – 1501 cm^{-1} region) : 0.791
KMO (for the 2500 – 2001 cm^{-1} region) : 0.861
KMO (for the 3000 – 2501 cm^{-1} region) : 0.838
KMO (for the 3500 – 3001 cm^{-1} region) : 0.847
KMO (for the 4000 – 3501 cm^{-1} region) : 0.888

Validation and Verification of Data Model

To achieve the relevant findings, pre-requisite MVDA must be carried out, beginning with choosing the appropriate dataset for the study using the KMO test [20]. The KMO test is an important step before conducting the PCA because it assesses the adequacy of the data for factor analysis by measuring the sampling adequacy for each variable. The KMO test also compares the magnitude of observed correlation coefficients to partial correlation coefficients. Meaning that if the variables have high partial correlations, it suggests that the correlations between variables are more likely due to change, thus the PCA will not be effective in summarising all the data; as in this context, all FTIR wavenumber large datasets. A high KMO value indicates that the correlations between variables can be explained by fewer underlying factors, which is essential for the PCA [21]. For the 728 datasets, the KMO test produced KMO scores in the range of 0.641 – 0.888 for all FTIR wavenumbers in the region of 4000 – 650 cm^{-1}, as depicted in **Table 1**. A lack of the KMO test may result in reporting inaccurate findings and interpretations due to small data set. Based on the general report, the KMO value of 0.8 – 0.9 is deemed good.

However, there is a lack in studies that mention any dataset transformation. Hence, this study recommends that all variables be transformed by using the standard deviation (n-1) method. Dataset transformation simultaneously corrects linearity issues as well. Hence, linearity test is on a case-by-case basis. The last step before the MVDA is performing the assumption testing, which involves normalisation. The normalisation of the dataset was conducted by performing the Shapiro-Wilk test at $\alpha = 0.01$, which allows only a 1% chance of false positive as this study is designed for authentication analysis. The Shapiro-Wilk test also helps determine if the data approximate a normal distribution, which enhances the validity of PCA by ensuring that the assumptions of linearity, variance, and interpretability of the PCA are met [22]. Thus, it should be very effective in reducing errors.

Principal Component Analysis (PCA)

Principal component analysis (PCA) was used as an unsupervised method to classify all the oil samples based on the correlation between the variables. Before the analysis, all the outliers were removed from the dataset. The KMO verified the sampling adequacy as a statistic, indicating the proportion of variance

underlying factors might cause in the variables. In the authentication application, KMO values (close to 1) indicate that factor analysis may be helpful for the data. Otherwise, with a value less than 0.5, the result of the factor analysis may not be beneficial [23]. At the same time, Bartlett's sphericity was used to test the hypothesis that the correlation matrix is an identity matrix to determine whether the variables are unrelated, and therefore are unsuitable for structure detection. Smaller than 0.05 significant level values indicate the usefulness of the factor analysis in the dataset. Noteworthy, when performing data pre-processing on the FTIR-ATR spectra for PCA, dividing the spectra into specific wavenumber ranges is often done for selective analysis of relevant regions [24]. This because certain wavenumber ranges correspond to specific vibrational modes of molecular bonds, such as C-H, O-H, and C=O. Thus, dividing the spectra allows the focus to be on regions that are most relevant to the compounds being studied, in this case the fatty acids; excluding the noise or irrelevant regions. Next, performing the data pre-processing helps reduce the dimensionality of the data, ensuring that only meaningful and interpretable variations are included in the PCA [25]. This can lead to more accurate and robust clustering/ classification results by removing irrelevant information.

Meanwhile, this research also provides a series of PCA plots for oils extracted from animals, as well as palm oil, which each focusing on a different region of the FTIR spectrum. Moreover, PCA is a powerful statistical technique used to reduce the dimensionality of complex datasets while retaining most of the variation. In this case, it is applied to FTIR spectral data to identify patterns and differences in the chemical composition of animal-derived oils. The FTIR spectroscopy is a widely used analytical method that provides information about the molecular structure and functional groups present in a sample. However, different regions of the FTIR spectrum correspond to different types of molecular vibrations, and consequently, different chemical functionalities. By applying PCA to specific regions of the FTIR spectrum and focusing more on particular chemical features impart better understanding of the similarities and differences among all the oil samples.

The percentage values in parentheses represent the total variance explained by the principal components shown in each plot. Noteworthy, scree plots are also presented in **Figure 4** to explain the percentage of variance associated with each principal component (PC) obtained by showing a graph between eigenvalues and PC numbers. Moreover, when PCA variance values are remarkably high, ranging from 74.75% to 98.79%, this indicates that the PCA has successfully captured most of the data's variability in each spectral region using just two or three principal components. To each PCA plot, each point represents an individual oil sample. Points that are close together have similar spectral features in that specific region, indicating similar chemical compositions. Points that are far apart have different spectral features, suggesting differences in chemical compositions.

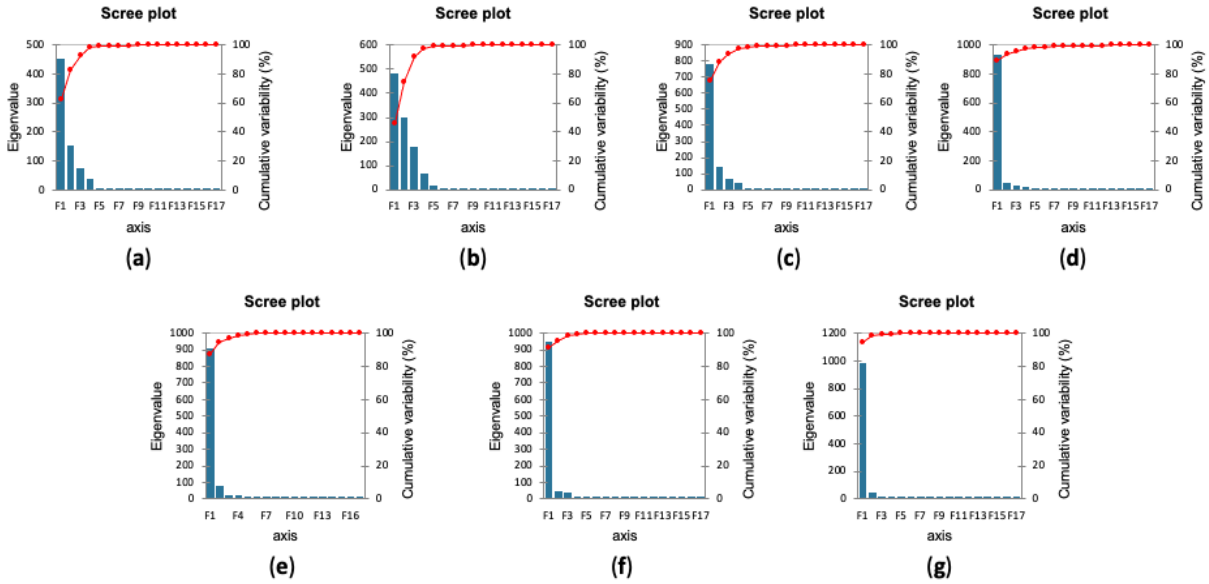

Figure 4. Scree plots after 728 dataset transformation of beef, chicken, pork, mutton, and palm oils FTIR spectra based on wavenumber regions of (a) 1000 – 650 cm^{-1}; (b) 1500 – 1001 cm^{-1}; (c) 2000 – 1501 cm^{-1}; (d) 2500 – 2001 cm^{-1}; (e) 3000 – 2501 cm^{-1}; (f) 3500 – 3001 cm^{-1}; and (g) 4000 – 3501 cm^{-1}.

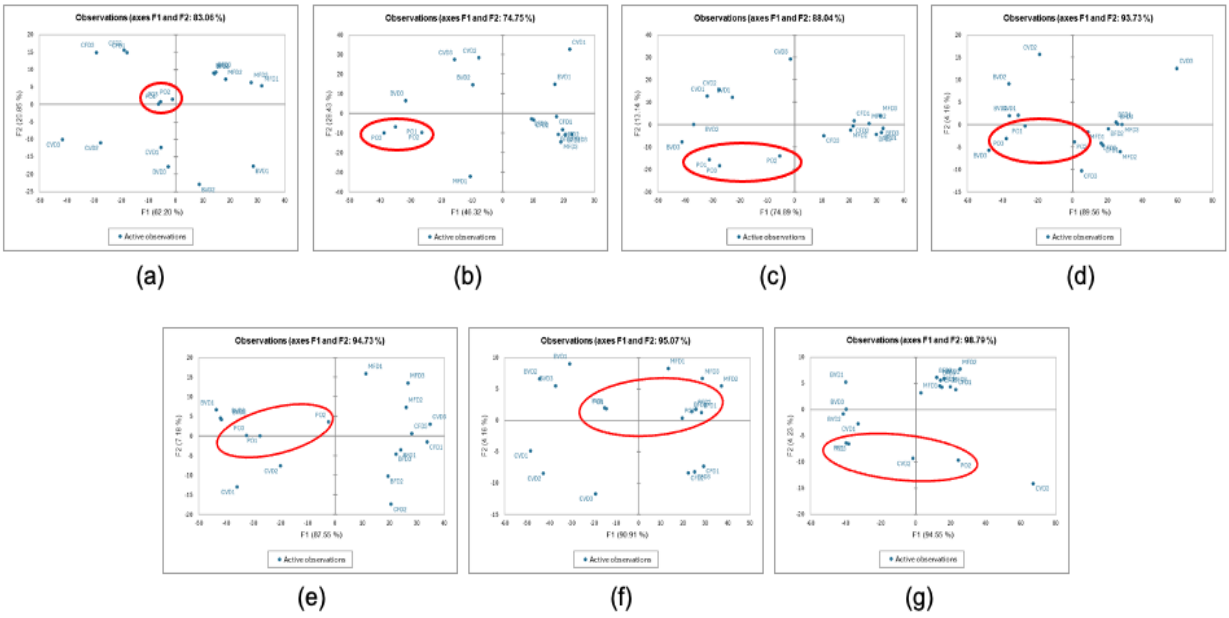

Figure 5. Principal component analysis (PCA) after 728 dataset transformation of beef, chicken, pork, mutton, and palm oils with the observation value ranging between 74.75 – 98.79% of FTIR wavenumber regions of (a) 1000 – 650 cm^{-1}; (b) 1500 – 1001 cm^{-1}; (c) 2000 – 1501 cm^{-1}; (d) 2500 – 2001 cm^{-1}; (e) 3000 – 2501 cm^{-1}; (f) 3500 – 3001 cm^{-1}; and (g) 4000 – 3501 cm^{-1}.

The high variance explained in each region suggests that there are indeed significant differences among the oil samples. For example, in the 4000–3501 cm^{-1} region, a staggering 98.79% of the variance is explained, indicating substantial differences in the presence and nature of O–H and N–H bonds. This could reflect differences in the fatty acid composition, such as the presence of hydroxy fatty acids or amino acids/peptides in the oil samples. Similarly, the high variance explained in the 2000 – 1501 cm^{-1} region (88.045%) suggests significant differences in unsaturated fatty acids (C=C bonds) and oxidation products (C=O bonds). This could indicate variations in the degree of unsaturation and oxidative stability among the oils. The region with the lowest explained variance is 1500 – 1001 cm^{-1} (74.75%), which still represents a substantial portion of the total variance.

This region's complexity, reflecting various functional groups, might contribute to its lower explained variance. The PCA analysis of FTIR data provides a rich, multifaceted view of the chemical differences among the animal-derived oils. By examining different spectral regions, we are able to infer differences in fatty acid composition, degree of unsaturation, oxidation state, and the presence of minor components like amino acids. This information is valuable for understanding the nutritional, functional, and stability properties of these oils, which can guide their use in food, cosmetic, or industrial applications. Further research could involve identifying the specific animals or diets that lead to these compositional differences [26].

Exploitation of PCA and PLS-DA for Halal Authentication of Oil Samples

Based on the analytical approach of the FTIR with the multivariate data analysis of PCA, *halal* authentication, a critical issue in the global food industry, is a particularly relevant context especially in regions with significant Muslim populations. In this context, all *halal* products must meet strict requirements, including the type of animal, for instance, no pigs, the method of slaughter, and the absence of any *haram* (forbidden) substances. Furthermore, authentication of *halal* status is not just a religious concern but also a matter of consumer trust, food safety, and ethical trade practices [27]. Next, the PCA analysis of FTIR spectral data provides a powerful tool for *halal* authentication of animal-derived oils. The FTIR spectroscopy offers a "molecular fingerprint" of a sample, reflecting its chemical composition. By applying PCA toward FTIR data, we can identify patterns and differences that might not be apparent from raw spectra alone. This is particularly useful in *halal* authentication, where the goal is to distinguish between permitted and forbidden animal products, or to detect any adulterants. The study shows PCA plots for seven different FTIR regions, each capturing a substantial portion of the data's variance (74.75 – 98.79%). This high explained variance suggests that there are indeed significant chemical differences among the oil samples.

There are factors that link the spectroscopic spectra with the multivariate data analysis within this research scope. Firstly, the FTIR fingerprint region

of 1000 – 650 cm^{-1} (83.06% variance explained) is particularly valuable for animal species identification. Each species has a unique molecular signature in this region. For *halal* authentication, this could help distinguish between oils from *halal* animals (beef, chicken, and goat) and *haram* animals such as pigs. The high variance explained suggests substantial differences, which is promising for accurate species identification. Next, due to fat composition of all the extracted oils, FTIR regions of 2000 – 1501 cm^{-1} (84.04% variance) and 3000 – 2501 cm^{-1} (94.73% variance) reflect differences in fatty acid composition. The former shows variations in unsaturated fats (C=C bonds), while the latter indicates differences in saturated fats (C-H bonds). Different animal species have distinct fat profiles, for instance, lard tends to be more unsaturated than beef fat. These spectral differences could help identify the animal source, supporting *halal* authentication. Also, the presence of adulterants of high variance in regions like 3500 - 3001 cm^{-1} (95.07%) and 4000 – 3501 cm^{-1} (98.79%), which show O–H and N–H bonds, could indicate the presence of adulterants. For example, some unethical producers might add cheaper, non-*halal* oils, likely lard, to *halal* oils. These adulterants could introduce different types of fatty acids, for instance, hydroxy fatty acids or minor components of different peptides, which are detectable in these spectral regions [28].

The power of this FTIR-PCA approach lies in its ability to provide a holistic view of the oil's chemical composition. Unlike targeted methods that look for specific compounds, this technique captures the overall molecular profile. This is crucial in *halal* authentication, where differences can be subtle, and adulterants might be chemically similar to genuine products. Moreover, the high variance explained in each region suggests that this method is sensitive enough to detect these subtle differences. For instance, the staggering 98.79% variance in the 4000 – 3501 cm^{-1} region indicates that even minor components, which might be key to distinguishing *halal* from *haram*, are captured [29,30]. However, it's important to note that while PCA-FTIR can flag differences, it doesn't automatically identify the cause. A sample that stands out in the PCA plot isn't necessarily *haram*; it's just chemically different. Further analyses, like mass spectrometry, chromatography or DNA testing, would be needed to confirm the exact compounds and their sources. Although FTIR-PCA is powerful in distinguishing animal fatty acids based on their respective functional and fingerprint groups' wavenumbers, nevertheless, some animal fats or adulterants may have very similar FTIR spectral patterns [31]. For example, the fatty acid composition of certain animal species might be similar enough that PCA struggles to differentiate between them without additional spectral resolution or pre-processing technique. Furthermore, the effectiveness of FTIR-ATR is highly dependent on the homogeneity of the sample. If the sample is not evenly mixed, or there are inconsistencies in the sampling process, PCA may misclassify or fail to detect adulterants due to variations in the spectral data. This is especially challenging when identifying specific adulterants dispersed unevenly in a matrix [32].

CONCLUSION

This study demonstrates the powerful synergy of FTIR-ATR and multivariate data analysis for rapid, non-destructive *halal* authentication of animal-derived oils. The PCA of the FTIR spectral data revealed distinct clustering patterns among beef, chicken, pork, mutton, and palm oil samples, with remarkably high total variance explained (74.75 – 98.79%) across different spectral regions. The fingerprint region (1000 – 650 cm^{-1}) proved particularly decisive for species identification, while regions associated with fatty acid composition (2000 – 1501 cm^{-1} and 4000 – 2501 cm^{-1}) showed promise in distinguishing *halal* from non-*halal* sources. Notably, the high variance in regions reflecting O–H and N–H bonds (4000 – 3501 cm^{-1}) suggests potential for detecting adulterants or minor components critical to *halal* status. This FTIR-ATR/MVDA approach offers a holistic view of oil composition, capturing subtle differences that might elude targeted methods, while providing the speed and cost-effectiveness necessary for industrial and regulatory applications. While additional confirmatory tests may be needed for definitive identification of non-*halal* components, this method represents a significant advancement in *halal* authentication technology. Its high sensitivity and specificity make it a promising tool for ensuring product integrity, maintaining consumer trust, and facilitating ethical trade practices in the rapidly growing global *halal* market. Future research should focus on expanding the sample set and integrating this approach with other analytical techniques for comprehensive *halal* authentication, potentially revolutionising quality control in the *halal* food industry.

CONFLICT OF INTEREST

All authors declare that they have no competing interests that could have appeared to influence the work reported in this research paper.

ACKNOWLEDGEMENT

All the authors humbly recognized Innovation Centre for Agritechnology for Advanced Bioprocessing (ICA), Universiti Teknologi Malaysia (Pagoh campus) for supporting and providing facilities as well this present work under the funding from UTM QuickWin Research [Grant No.: R.K130000.7743.4J616] and UTM Fundamental Research [No.: Q.K130000.3842. 21H95] grants. The research also has been done in the facilities of Food Analysis Laboratory, Innovation Centre for Agritechnology for Advanced Bioprocessing (ICA), Universiti Teknologi Malaysia (Pagoh Campus).

REFERENCES

1. Ahmad, A. N., Abidin, U. F. U. Z., Othman, M. & Rahman, R. A. (2018) Overview of the Halal Food Control System in Malaysia. *Food Control*, **90**, 352–363.

2. Wiyono, S. N., Deliana, Y., Wulandari, E. & Kamarulzaman, N. H. (2022) The Embodiment of Muslim Intention Elements in Buying Halal Food Products: A Literature Review. *Sustainability*, **14(20)**, 13163.

3. Abdullah, W. N. W., Yang, T. A., Sifat, S. A. D. & Zzaman, W. (2021) Development of Safe Halal Food Management System (SHFMS). *Food Control*, **127**, 108137.

4. Vanany, I., Soon, J. M., Maryani, A. & Wibawa, B. M. (2020) Determinants of Halal-Food Consumption in Indonesia. *Journal of Islamic Marketing*, **11(2)**, 507–521.

5. Vizano, N. A., Khamaludin, K. & Fahlevi, M. (2021) The Effect of Halal Awareness on Purchase Intention of Halal Food: A Case Study in Indonesia. *The Journal of Asian Finance, Economics and Business*, **8(4)**, 441–453.

6. Kua, J. M., Azizi, M. M. F., Abdul Talib, M. A. & Lau, H. Y. (2022) Adoption of Analytical Technologies for Verification of Authenticity of Halal Foods – A Review. *Food Additives & Contaminants: Part A*, **39(12)**, 1906–1932.

7. Aziz, A. A., Nordin, F. N. M., Zakaria, Z. & Abu Bakar, N. K. (2022) A Systematic Literature Review on the Current Detection Tools for Authentication Analysis of Cosmetic Ingredients. *Journal of Cosmetic Dermatology*, **21**(1), 71–84.

8. Ng, P. C., Ahmad Ruslan, N. A. S., Chin, L. X., Ahmad, M., Abu Hanifah, S., Abdullah, Z. & Khor, S. M. (2022) Recent Advances in Halal Food Authentication: Challenges and Strategies. *Journal of Food Science*, **87(1)**, 8–35.

9. Windarsih, A., Indrianingsih, A. W., Apriyana, W. & Rohman, A. (2023) Rapid Detection of Pork Oil Adulteration in Snakehead Fish Oil using FTIR-ATR Spectroscopy and Chemometrics for Halal Authentication. *Chemical Papers*, **77(5)**, 2859–2870.

10. Abidin, S. A. S. Z., Nizar, M., Nizar, N. N. A., Shukor, M. S. A., Zainal, R. & Ab Mutalib, S. R. (2023) Halal Concerns on Lard in Food Products and its Detection Methods. *Halalsphere*, **3(2)**, 79–90.

11. Rohman, A. & Windarsih, A. (2020) The Application of Molecular Spectroscopy in Combination with Chemometrics for Halal Authentication Analysis: A Review. *International Journal of Molecular Sciences*, **21(14)**, 5155.

12. Ahda, M. & Guntarti, A. (2023) Halal Food Analysis using FTIR Spectroscopy Combined with Chemometrics: A Review. *Current Nutrition & Food Science*, **19(2)**, 125–135.

13. Nizar, N. A., Abidin, S. A. S. Z., Abd Ghafur, A. N. S., Bujang, A. & Jailani, N. F. (2022) Fourier Transform Infrared (FTIR) Spectroscopy and Physicochemical Analysis for Authentication of Olive Oil Adulterated with Sunflower Oil. *al-Qanatir: International Journal of Islamic Studies*, **28**(2), 92–103.

14. Ahda, M., Guntarti, A., Kusbandari, A. & Nugroho, H. A. (2023) Identification of Adulterated Sausage Products by Pork using FTIR and GC-MS Combined with Chemometrics. *Journal of Chemical Health Risks*, **13**.

15. Nebolisa, N. M., Umeyor, C. E., Ekpunobi, U. E., Umeyor, I. C. & Okoye, F. B. (2023) Profiling the Effects of Microwave-Assisted and Soxhlet Extraction Techniques on the Physicochemical Attributes of Moringa Oleifera Seed Oil and Proteins. *Oil Crop Science*, **8(1)**, 16–26.

16. Addinsoft, Addinsoft (2019) XLSTAT Statistical and Data Analysis Solution. *Long Island, NY, USA*. https://www.xlstat.com.

17. Behzadi, S., Akbarzadeh Chamchaei, G., Momeni, M., Salarpouri, A., Darvishi, M., Mohebi Nouzar, S. & Mortazavi, M. S. (2019) Assessment of the Effect of Climate Parameters in Commercial Shrimp Fishing using Principal Components Analysis (PCA) (Hormozgan Province). *Journal of Animal Environment*, **11(2)**, 347–352.

18. Schreiber, J. B. (2021) Issues and Recommendations for Exploratory Factor Analysis and Principal Component Analysis. *Research in Social and Administrative Pharmacy*, **17(5)**, 1004–1011.

19. González-Estrada, E. & Cosmes, W. (2019) Shapiro–Wilk Test for Skew Normal Distributions based on Data Transformations. *Journal of Statistical Computation and Simulation*, **89(17)**, 3258–3272.

20. Ordoudi, S. A., Strani, L. & Cocchi, M. (2023) Toward the Non-Targeted Detection of Adulterated Virgin Olive Oil with Edible Oils via FTIR Spectroscopy & Chemometrics: Research Methodology Trends, Gaps and Future Perspectives. *Molecules*, **28(1)**, 337.

21. Yuan, L., Meng, X., Xin, K., Ju, Y., Zhang, Y., Yin, C. & Hu, L. (2023) A Comparative Study on Classification of Edible Vegetable Oils by Infrared, Near Infrared and Fluorescence Spectroscopy Combined with Chemometrics. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **288**, 122120.

22. Sapian, N. A. S., Roslan, M. A. M., Hashim, A. M., Desa, M. N. M., Halim, M., Manaf, Y. N. A. & Wasoh, H. (2023) Differentiation of Lard from Other Animal Fats based on *n*-Alkane Profiles using Chemometric Analysis. *Food Research International*, **164**, 112332.

23. Kharbach, M., Alaoui Mansouri, M., Taabouz, M. & Yu, H. (2023) Current Application of Advancing Spectroscopy Techniques in Food Analysis: Data Handling with Chemometric Approaches. *Foods*, **12(14)**, 2753.

24. Dayananda, B., Owen, S., Kolobaric, A., Chapman, J. & Cozzolino, D. (2023) Pre-Processing Applied to Instrumental Data in Analytical Chemistry: A Brief Review of the Methods and Examples. *Critical Reviews in Analytical Chemistry*, 1–9.

25. Hategan, A. R., David, M., Dehelean, A., Cristea, G., Puscas, R., Molnar, A. J. & Magdas, D. A. (2023) Impact of Pre-Processing Methods for the Identification of the Botanical Origin of Honey Based Upon Isotopic and Elemental Profiles. *Analytical Letters*, **56(2)**, 231–243.

26. Gour, L., Maurya, S. B., Koutu, G. K., Singh, S. K., Shukla, S. S. & Mishra, D. K. (2017) Characterization of Rice (*Oryza sativa L.*) Genotypes using Principal Component Analysis including Scree Plot & Rotated Component Matrix. *International Journal of Chemical Studies*, **5(4)**, 975–983.

27. Wang, H. P., Chen, P., Dai, J. W., Liu, D., Li, J. Y., Xu, Y. P. & Chu, X. L. (2022) Recent Advances of Chemometric Calibration Methods in Modern Spectroscopy: Algorithms, Strategy, and Related Issues. *TrAC Trends in Analytical Chemistry*, **153**, 116648.

28. Dupont, M. F., Elbourne, A., Cozzolino, D., Chapman, J., Truong, V. K., Crawford, R. J. & Latham, K. (2020) Chemometrics for Environmental Monitoring: A Review. *Analytical Methods*, **12(38)**, 4597–4620.

29. Nurani, L. H., Riswanto, F. D. O., Windarsih, A., Edityaningrum, C. A., Guntarti, A. & Rohman, A. (2022) Use of Chromatographic-Based Techniques and Chemometrics for Halal Authentication of Food Products: A Review. *International Journal of Food Properties*, **25(1)**, 1399–1416.

30. Razak, N. F., Abd Karim, R. H., Jamal, J. A. & Said, M. M. (2020) Rapid Discrimination of Halal and Non-Halal Pharmaceutical Excipients by Fourier Transform Infrared Spectroscopy and Chemometrics. *Journal of Pharmacy and Bioallied Sciences*, **12(Suppl 2)**, S752–S757.

31. Windarsih, A., Bakar, N. K. A., Dachriyanus, Yuliana, N. D., Riswanto, F. D. O. & Rohman, A. (2023) Analysis of Pork in Beef Sausages Using LC-Orbitrap HRMS Untargeted Metabolomics Combined with Chemometrics for Halal Authentication Study. *Molecules*, **28(16)**, 5964.

32. Suratno, Windarsih, A., Warmiko, H. D., Khasanah, Y., Indrianingsih, A. W. & Rohman, A. (2023) Metabolomics and Proteomics Approach using LC-Orbitrap HRMS for the Detection of Pork in Tuna Meat for Halal Authentication. *Food Analytical Methods*, **16(5)**, 867–877.