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Research Article

Preliminary analysis of standard animal gelatines through FTIR-ATR spectroscopy coupled with multivariate data analysis and pattern recognition for halal verification

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Abstract
The global halal market faces increasing challenges regarding product authenticity, particularly issues of contamination, adulteration, and fraudulent trading practices across Muslim and non-Muslim countries. Authenticating halal status in food, pharmaceutical, and cosmetic products has become crucial for ensuring compliance with Islamic dietary laws and maintaining consumer trust. While various analytical techniques, including high-perfomance liquid chromatography (HPLC), gas chromatography-flame ionization detection (GC-FID), gas chromatography-mass spectrometry (GC-MS), and nuclear magnetic resonance (NMR) spectroscopy, have been employed for halal verification, there remains a need for rapid, cost-effective, and reliable analytical methods that can be applied in a standardised manner. Herein, this study presents an approach utilizing Fourier transform infrared apectroscopy with attenuated total reflectance (FTIR-ATR) to differentiate and authenticate the halal status of standard gelatine from fish, bovine, and porcine sources. Spectral wavenumber data acquired in the 4000 – 650 cm^{-1} region were collected and analysed using discriminant (DA) and principal component (PCA) analyses for pattern recognition and classification. The DA revealed high discriminating power, particularly in the 2000 – 1501 cm^{-1} region, achieving 100% accuracy in training and 83.3 – 100% in cross-validation, clearly distinguishing between halal (fish and bovine) and non-halal (porcine) standard gelatine samples. The PCA further validated these findings, with the Cumulative Variability (CV) of PCs explaining up to 99.99% of the total variance and demonstrating a clear separation of gelatine standards in biplots. The combined approach effectively established unique spectral fingerprints, enabling accurate classification of halal products. Therefore, this method provides a robust, real-time, and cost-effective tool for halal verification, with strong potential for industrial implementation and regulatory applications.

Keywords: animal gelatine, FTIR-ATR spectroscopy, multivariate data analysis, pattern recognition, halal verification

Introduction

Gelatine is a type of biopolymer of animal origin produced through the partial hydrolysis of collagen [1]. Moreover, gelatine is composed of 19 amino acids, with glycine (27–35%), proline, and hydroxyproline (20–24%) being the most abundant in terms of composition [2]. The types of gelatine are distinguished by their production methods and the unique properties they possess [3]. Type A gelatine is derived from collagen processed with acid, whereas Type B gelatine is obtained from collagen treated with alkali, as presented in **Figure 1**. Commercially,

gelatine has traditionally been derived from mammalian tissues, such as the skin and bones of bovine, porcine, or caprine species. However, there is growing interest in sourcing gelatine from alternative sources, particularly marine organisms, to mitigate risks like bovine spongiform encephalopathy and swine flu and address religious considerations [4]. In food and pharmaceutical applications, gelatine plays a key role in stabilizing emulsions and foams, maintaining a uniform texture, and preventing the separation of ingredients, making it valuable in products such as whipped cream [5]. Gelatine is also added to enhance the viscosity of products, improving the mouthfeel and consistency of items like soups, sauces, and dairy products [6]. Given the widespread use of gelatine in food products and the diverse sources it can be derived from, ensuring its halal certification is critical, especially for Muslim consumers.

Halal, meaning permissible and lawful, is an essential aspect of Syariah Law in the Islamic teachings [8]. Noteworthy, halal and kosher dietary laws share similarities, but their sources and requirements differ. Halal rules come from the al-Quran and Hadith, while kosher laws are based on the Torah and Talmud [9]. A key difference between halal and kosher animal slaughter is that halal requires the animal to be alive, while kosher law requires it to be both alive and conscious. The following is the slaughtering process, where kosher animals must undergo shechita, a specific process carried out by a trained shochet using a "chalef" knife. Dietary principles and kosher laws are based on four principles: prohibiting blood consumption and restricting dairy and meat mixing. Both halal and kosher laws prohibit pork, but kosher law also prohibits shellfish and rabbits [8]. Since both halal and kosher dietary laws share similarities, such as prohibiting pork, the need to ensure the halal status of food products such as gelatine is vital in areas with large Muslim populations, such as Malaysia and Indonesia, respectively. In Malaysia, the halal status of gelatine has become controversial due to its animal origins. A significant portion of gelatine in the market is derived from animals that are not permissible for Muslim consumption, such as porcine gelatine. Bahar and co-workers reported that pig skin accounts for approximately 46% of gelatine production, followed by bovine skin and bones at 29.4% and 23.1%, respectively, with other sources contributing around 1.5% [10]. In addition to the concerns over animal origins, cases of mislabelling have been identified. Experimental findings by Sultana and co-workers revealed that four of the 35 products tested were mislabelled in terms of their gelatine or gelling agent content [11]. Specifically, Firm Up and Lenox Collagen were labelled as containing fish-derived ingredients but were also reported to have included bovine materials, as indicated by Ct values of 30.22 ± 0.12 and 29.34 ± 0.14, respectively. Jellybeans and Yupi Gummi Pizza also tested positive for porcine species, with Ct values of 30.12 ± 0.22 and 28.02 ± 0.02, as demonstrated in **Figure 2**, respectively.

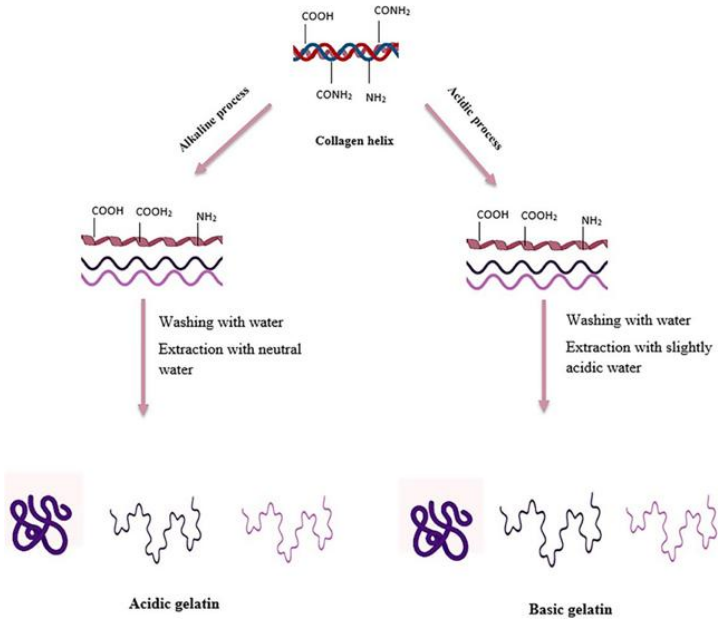

Figure 1. Gelatine synthesis from collagen under acidic or alkaline conditions, according to Teimouri and co-workers [7]

Table 1. Analysis of dietary supplements, yogurt and processed foods using an optimized multiplex qPCR system by Sultana and co-workers [11]

Sample Information				DNA		PCR Results				
#	Product Description on Label	Origin	Gelatin Type	Concentration (ng/µL)	Purity (A260/280)	Porcine (Ct value)	Bovine (Ct value)	Fish (Ct value)	%PCR Efficiency	No. of Mislablled
				Edible gelatine powder						0/2
1	Halal gel	Malaysia	Beef	32	1.8	-	26.12 ± 0.07	-	100	
2	David gelatin powder	China	Beef	25	1.9	-	26.18 ± 0.03	-	100	
				Dietary supplements						2/4
1	Kinohimitsu collagen Men	Malaysia	Fish	20	1.8	-	-	27.26 ± 0.05	100	
2	Kinohimitsu Diamond	Malaysia	Marine fish	17	1.9	-	-	30.53 ± 0.06	100	
3	Kinohimitsu Nite 16 s	Malaysia	Fish	18	1.9	-	-	-	-	
4	Firm Up	Malaysia	Marine fish	18.5	1.8	-	30.22 ± 0.12	29.02 ± 0.03	100	
5	Lenox Collagen	Malaysia	Fish	32	1.9	-	29.34 ± 0.14	28.134 ± 0.08	100	
				Yogurt						0/5
1	Vitagen Cultured drink	Malaysia	-	18.5	1.8	-	-	-	-	
2	Vitagen Collagen drink	Malaysia	Fish	23	1.8	-	-	29.354 ± 0.12	-	
3	Calpis	Malaysia	Plant	14	1.9	-	-	-	-	
4	Nestle yogurt	Malaysia	-	23	1.8	-	33.34 ± 0.40	-	-	
5	Nestle sour cream	Malaysia	-	18	1.8	-	29.32 ± 0.34	-	-	
				Gummy						
1	Gummy pizza	Indonesia	Beef gelatin	23	1.9	-	32.12 ± 0.33	-	100	
2	Starburst babies	Australia	Gelatin (Halal)	34	1.8	-	33.23 ± 0.1	-	100	
3	Yupi gummy pizza	Indonesia	Gelatine (Bovine)	25	1.8	29.32 ± 0.34	-	-	100	
4	HARIBO sour S' ghetto	Turkiye	Beef gelatin	24	1.9	-	29.06 ± 0.3	-	100	
5	Darry's strawberry gummy	Malaysia	Gelatin (bovine)	21	1.8	-	31.43 ± 0.27	-	100	
6	Lot100 happy mix gummy	Malaysia	Gelatin (bovine)	19	1.9	-	32.17 ± 0.34	-	100	
7	HARIBO cola bear	Turkiye	Beef gelatin	26	1.8	-	29.24 ± 0.42	-	100	
8	Sour + Cocoaland	Malaysia	Bovine gelatin	28	1.8	-	28.34 ± 0.53	-	100	
9	Cocoland strawberry candy	Malaysia	Beef gelatin	26	1.7	-	29.21 ± 0.12	-	100	
10	Snaker	Australia	Gelatin	18	1.8	-	33.34 ± 0.22	-	100	

Traditional methods for detecting non-halal additives, such as physico-chemical methods, are cumbersome and unreliable. Physical attributes such as texture and colour can help distinguish porcine meat from other types of meat. However, these methods require intact anatomical features, making them unsuitable for identifying lean meat [12]. Chemical methods, while time-consuming, lack specificity, often failing to distinguish target markers from similar compounds [13]. These aforementioned methods often fail to provide accurate and reliable results despite the need for more advanced

techniques, such as Fourier transform infrared with attenuated total reflectance (FTIR-ATR) spectroscopy coupled with multivariate data analysis (MVDA), to ensure accurate halal verification. Advanced techniques like FTIR-ATR spectroscopy and MVDA provide fast and reliable solutions for verifying food authenticity, including halal status. The FTIR-ATR examines the molecular composition of materials by measuring their absorption of infrared light (**Figure 3**) [14]. It analyses the infrared region of the electromagnetic spectrum, which has longer wavelengths and lower frequencies than visible light. The technique determines a sample's molecular structure by exposing it to infrared radiation and measuring the specific wavelengths of light absorbed by atomic bonds at both functional and fingerprint wavenumbers [15]. The IR spectrum is divided into three regions: near-infrared (NIR, 14000 - 4000 cm^{-1}), mid-infrared (MIR, 4000 - 400 cm^{-1}), and far-infrared (FIR, 400 - 10 cm^{-1}) [16]. The most widely used method for food analysis is infrared spectroscopy, particularly in the NIR (14000 - 400 cm^{-1}) and MIR range (4000 - 400 cm^{-1}) [14].

In halal verification, FTIR-ATR is used to analyze the chemical composition of gelatine or other animal-based products. The raw spectral data from FTIR-ATR can contain complex patterns related to specific molecules, such as gelatine, typical of different animal sources. Pattern recognition techniques are employed to interpret these spectral data, enabling accurate predictions about whether a sample complies with halal standards [18-19]. The MVDA is a collection of statistical methods designed to simultaneously examine data with multiple variables. These techniques help uncover complex relationships between variables, making it easier to identify patterns, trends, and connections in the data [20]. Yudha and co-workers highlighted the usefulness of MVDA in halal authentication, particularly for analyzing complex datasets to ensure compliance with halal standards [21]. By applying several methods of MVDA, such as principal component (PCA) and discriminant (DA) analyses, researchers can classify gelatine samples into halal and non-halal categories, ensuring compliance with halal standards. The PCA is a dimensionality reduction method commonly used to simplify large datasets by transforming many variables into a smaller set that retains most of the essential information from the original dataset [22]. For instance, Rohman and Fadzillah utilized PCA to classify meat products as halal or non-halal based on their chemical composition. Their findings demonstrated that chemical profiles could effectively distinguish between the two categories, emphasizing the systematic approach of MVDA in evaluating food authenticity [23]. Next, DA classifies data into predefined groups by creating a hyperplane that minimizes misclassification errors, distinguishing halal from non-halal products based on chemical markers like metabolites and fatty acids [24-25]. A notable example of DA application in differentiating halal from non-halal products is the research performed by Mahmood and co-workers, where they used FTIR spectroscopy data to classify beef burgers. The researchers employed linear discriminant analysis (LDA) to develop a model distinguishing between halal and non-halal samples [26]. The results demonstrated that DA successfully classified most beef burger samples into halal and non-halal groups based on FTIR spectral data, with certain fatty acid profiles identified as key markers in the classification process. Hitherto, combining the FTIR-ATR spectroscopy with MVDA and pattern recognition provides a robust framework for gelatine halal verification consisting of bovine, porcine, and fish. This comprehensive approach addresses the limitations of traditional methods, enhancing the accuracy and reliability of halal certification processes and aligning with the global demand for transparent and trustworthy halal labelling especially gelatine products.

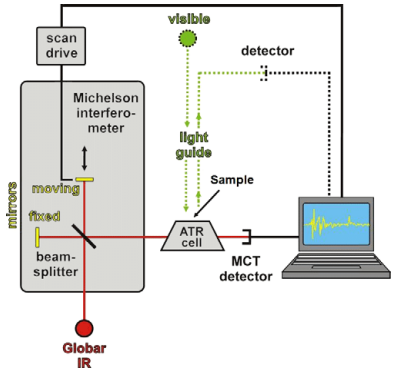

Figure 2. The schematic diagram of how FTIR-ATR works by Jain and Agarwal [17].

Materials and Methods

Experimental design

The current study utilised gelatine from fish, bovine, and porcine sources via FTIR-ATR spectroscopy. All the datasets were subjected to pre-processing, dataset transformation, DA, and PCA, as illustrated in the experimental design section of **Figure 4**.

Materials

Three gelatine samples were used in this study, comprising standard gelatine of fish (n = 1) (Sigma-Aldrich, USA), bovine (n = 1) (Sigma-Aldrich, USA), and porcine (n = 1) (Sigma-Aldrich, USA). All samples were commercially available, standard, and reference-grade material to ensure purity. These samples were selected as representative models of distinct gelatine sources for preliminary comparative analysis. Ethanol (R&M Chemicals, UK) was analytical grade and used as received.

Colour analysis

All the gelatines were analysed by colour and clarity using a colorimeter (Hisuc, China). The analysis of the colour and clarity was based on Commision Internationale de l'Éclairage (CIE) of L* for lightness, a* for redness or greenness, and b* for yellowness or blueness of the colour system.

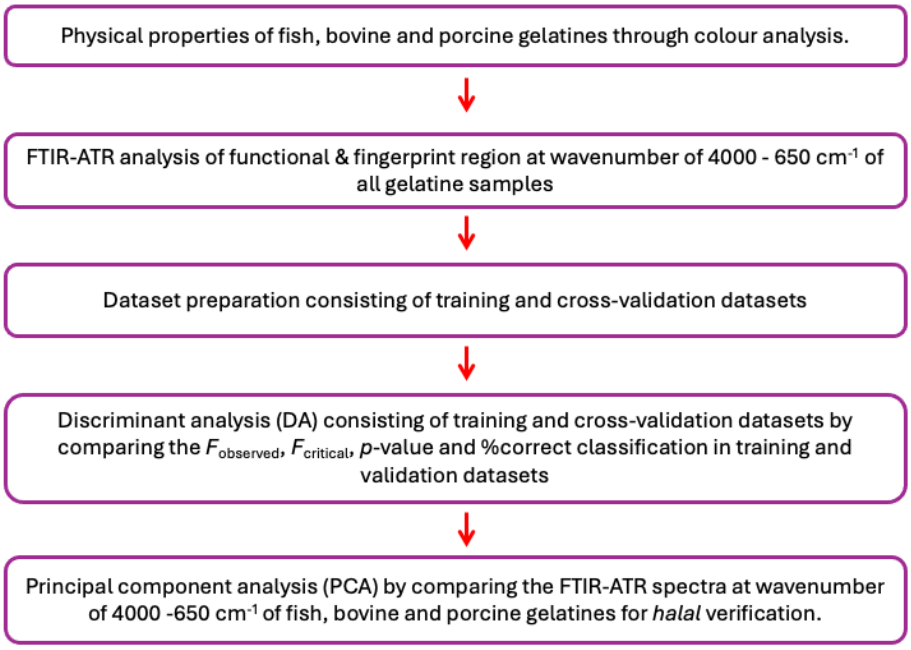

Figure 3. The experimental design is presented through a flow chart for the current research study

The FTIR-ATR measurement

The Nicolet iS5 spectrophotometer model (Thermo Scientific, USA), equipped with an ATR accessory featuring a diamond cell, was used for the measurements. Spectra were recorded in the 4000 – 650 cm-1 spectral range with a resolution of 4 cm^{-1}, with 32 scans per measurement. Three replicate spectra were collected from every gelatine sample of fish, bovine, and porcine for independent experiments to support multivariate analysis. All measurements were conducted under an ambient room temperature of 25°C. A single-beam spectrum was obtained for each sample, and the background of air spectra containing CO_2 was subtracted to present the result in transmittance units. Sample spectra were then recorded in triplicate and proceeded with the OMNIC software provided by Thermo Scientific.

Dataset pre-processing

The FTIR spectra were compiled into a Comma-Separated Values (CSV) format file in Microsoft Excel and exported into the XLSTAT software [27]. This pre-processing step consists of outlier treatment, dataset transformation, and a dataset adequacy test of $\alpha = 0.01$. The transmittance of the spectrum was separated into four different wavenumber ranges, i.e., 4000 – 3001 cm^{-1}, 3000 – 2001 cm^{-1}, 2000 – 1001 cm^{-1}, and 1000 – 650 cm^{-1}. Then, DA was carried out on all FTIR wavenumbers ranging from 4000 – 650 cm^{-1}. The outlier identification was carried out using the Grubbs and Dixon tests. To ensure normal distribution, the normality of all FTIR spectra was subjected to dataset transformation using the standardised (n–1) method. Subsequently, the Kaiser-Meyer-Olkin (KMO) test was used to verify the

dataset's adequacy before carrying out the DA with the combined wavenumber. The most significant wavenumbers were selected from the DA, and PCA was performed to determine the apportionment of wavenumbers in the gelatine samples.

The Kaiser-Meyer-Olkin (KMO) test

All the pre-processing datasets were analysed for dataset adequacy using the KMO test. An adequate dataset enables the generation of a model that extracts latent variables from the dataset. This study employed the KMO test at a significant level, α = 0.01. The calculated KMO was ranked as KMO < 0.5 = inadequate, 0.5 < KMO < 0.7 = passable, 0.7 < KMO < 0.8 = good, 0.8 < KMO < 0.9 = very good and KMO > 0.9 = excellent to indicate the dataset adequacy.

Dataset transformation

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

The discriminant analysis

The Discriminant Analysis (DA) established a discriminating model for all gelatine samples using the FTIR spectra database. A new column labelled 'cluster' was added to the training cross-validation datasets, and samples were assigned as "mammalian and marine sources," respectively. The DA was executed at α = 0.01 via this equation (1) in the form:

$$E_1 = f_0 + f_1g_1 + f_2g_2 + \ldots + f_ig_i, \quad (1)$$

to represent a linear model that combines the spectral features to discriminate between different classes. E_1 represent the discriminant score of the samples' FTIR spectra; f_0, is a constant, while f_1, f_2 and f_i are coefficients associated with each spectral feature of the wavenumber 4000 – 650 cm^{-1} and g_1, g_2, and g_i are the spectral features based on the intensities at specific wavenumbers of all the samples.

The F-statistics of $F_{observed}$ and $F_{critical}$ and the p-value of the Wilks' Lambda test were computed, and the individual F-statistics and p-value of significant FTIR-ATR spectra were determined. The correct classification of the clusters was examined using the training and cross-validation datasets, and the dissimilarities among fish, bovine, and porcine gelatines were explored. The clusters' Fisher distance value and p-value were also calculated to verify cluster dissimilarity.

The principal component analysis (PCA)

The entire FTIR spectrum was extracted from its transmittance value to obtain a dataset for PCA. The FTIR spectra at the combination wavenumbers, i.e., 4000 – 3001 cm^{-1}, 3000 – 2001 cm^{-1}, 2000 – 1001 cm^{-1}, and 1000 – 650 cm^{-1}, are used to build the PCA model because they reveal clear differences for authentication purposes. Analysis of PCA was performed using XLSTAT software (2024 version, France), and the data were scaled using the Pareto scaling technique prior to PCA analysis to maximize variation. A Pearson correlation of PCA at α = 0.01 was employed to group the FTIR spectra of fish, bovine and porcine gelatine distributions. The significance of FTIR spectra ($p<0.01$) identified by discriminant analysis was transformed and underwent PCA to generate the Principal Components (PCs) as the independent variables. The component score M for s PC number and n sample number can be expressed as equation (2):

$$M_{pr} = s_{p1}q_{r1} + s_{p2}q_{r3} + \ldots + s_{pi}q_{ri}, \quad (2)$$

where M_{pr} is the scores that represent the projection of the original data by the variance points onto the PCs, s_{p1} is the coefficients that represent the contribution of each original variable of spectral features/wavenumbers to PC, q_{r1} are the new axes or directions in the transformed space that capture the maximum variance in the data as it is orthogonal to each other and are linear combinations of the original spectral variables. Cumulative Variability (CV) of two-dimensional PCs, entailing PC1 and PC2, was computed for the FTIR spectra exploratory. Then, after Pareto scaling, the variables used for the PCA model were more normally distributed, as depicted by their Gaussian curve. The number of PCs was optimized for optimum sample differentiation. The differentiation result of the samples was observed using a PCA score plot. Moreover, the PCA model was evaluated using its R^2 and Q^2 values to justify the good fitness and predictivity of the PCA model, respectively.

Results and Discussion

Colour and clarity of the gelatine

The colorimetric analysis of gelatin samples derived from bovine, fish, and porcine sources reveals distinct variations in their optical properties as measured using the L*, a*, and b* colour space systems. Although colour values cannot independently verify the source or authenticity of the gelatine, they offer a descriptive parameter for standard sample profiling. However, this visual data may still be useful in quality control settings or as supplementary information when interpreting more robust analytical results. Additionally, colour analysis

provides a preliminary visual differentiation of samples, which is particularly relevant in consumer perception, especially in halal authentication, where appearance can influence acceptances. Noteworthy, the L* value, which indicates lightness (0 = black, 100 = white), depicts that porcine gelatin exhibits the highest lightness value at 83.03 ± 0.67, followed closely by bovine gelatin at 82.58 ± 12.01, while fish gelatin demonstrates the lowest lightness at 79.86 ± 3.09. Notably, the bovine gelatin reveals considerable variability in its lightness value, as indicated by its high standard deviation of 12.01, suggesting potential inconsistencies in its production process or raw material quality.

Moreover, the a* value, representing the red-green spectrum (positive values indicate redness, and negative values indicate greenness), demonstrates an interesting progression across the sources. Porcine gelatin exhibits the highest redness value at 3.89 ± 0.05, followed by fish gelatin at 2.99 ± 0.28, while bovine gelatin indicates minimal redness at 0.90 ± 0.06. This variation in redness could be attributed to differences in the collagen composition and processing conditions specific to each source material. The b* value, indicating the yellow-blue spectrum (positive values indicate yellowness, negative values indicate blueness), outlines that porcine gelatin possesses the highest yellowness at 14.72 ± 0.12, followed closely by fish gelatin at 13.59 ± 0.45, while bovine gelatin demonstrates significantly lower yellowness at 8.31 ± 0.07. The distinct yellowness in porcine and fish gelatines might be related to their specific protein compositions and various chromophores formed during the extraction and processing stages.

These colorimetric differences among gelatin sources could have significant implications for their applications in various industries, particularly in products where visual appearance is crucial, such as food and pharmaceutical applications. As indicated by the standard deviations, consistency in colour parameters might also influence quality control processes and product standardization efforts.

The FTIR-ATR analysis

The FTIR-ATR spectroscopy analysis revealed sufficient characteristic absorption bands that confirm the molecular structures and the functional groups in bovine, fish and porcine gelatines, respectively. The prominent absorption band observed in the region of 3300 – 3276 cm^{-1} corresponds to the N–H stretching vibrations and the hydrogen-bonded O–H groups, characteristic of protein structure [28]. This broad peak indicates the presence of intermolecular hydrogen bonding, which plays a crucial role in maintaining the triple-helical structure of the gelatine. The slight variations in peak positions among different gelatine sources of bovine at 3292 – 3285 cm^{-1}, fish at 3296 – 3278 cm^{-1} and porcine at 3300 – 3276 cm^{-1} suggest subtle differences in their hydrogen bonding networks.

Herein, the most distinctive spectral pattern for halal verification lies in the Amide I and Amide II regions. In the Amide I region, the porcine gelatine demonstrates a characteristic peak at 1647 cm^{-1}, while the halal animal source of bovine and fish illustrates lower wavenumbers of bovine at 1635 cm^{-1} and fish at 1645 cm^{-1}. This shift to higher wavenumbers in porcine gelatine indicates differences in the C=O stretching vibrations, possibly due to variations in the protein's secondary structure and the degree of hydrogen bonding [29]. Similarly, in the Amide II region, the porcine gelatine exhibits a distinctive peak at 1559 cm^{-1} compared to bovine at 1540 cm^{-1} and fish at 1537 cm^{-1}. These differences in Amide II bands reflect variations in the N–H bending and C–N stretching vibrations, which are influenced by the specific amino acid sequence and the molecular arrangement of each animal species [30].

Table 2. The colour analysis of the fish, bovine, and porcine samples

Gelatine	L	a*	b*
Fish	79.86 ± 3.09	2.99 ± 0.28	13.59 ± 0.45
Bovine	82.58 ± 12.01	0.90 ± 0.06	8.31 ± 0.07
Porcine	83.03 ± 12.01	3.87 ± 0.05	14.72 ± 0.12

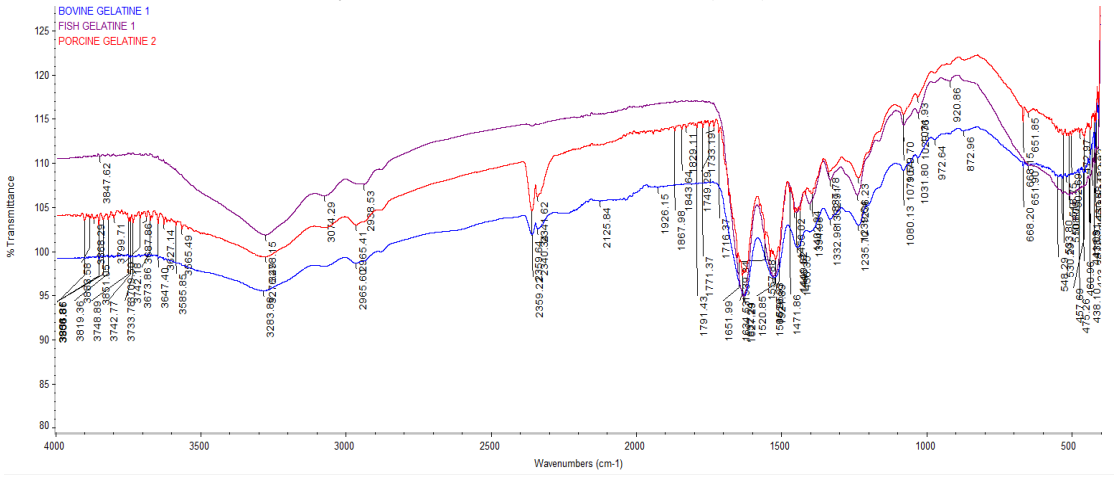

Figure 4. The overlay FTIR-ATR spectra of the fish, bovine, and porcine gelatines on the wavenumber range of 4000 – 650 cm^{-1}

Table 3. Literature review of the FTIR-ATR wavenumber spectral interpretation [31-32]

Fish Gelatine	Bovine Gelatine	Porcine Gelatine	Functional Group
3296	3292	3300	N–H stretching
1645	1635	1647	C=O stretching
1537	1540	1559	Bending vibration N–H group, stretching
1448	1442	-	vibration of C–N group –CH_2 (symmetric bending)
1238	1231	-	C–N stretching and N–H deformation
1021	1035	1034	–OH groups, C–O stretching

Table 4. Experimental data of the FTIR-ATR wavenumber spectral interpretation

Fish Gelatine	Bovine Gelatine	Porcine Gelatine	Functional Group
3278	3285	3276	Hydroxyl group, H-bonded O–H stretch
1632	1627	1635	C=O stretching
1530	1522	1521	Bending vibration N–H group, stretching
1450	1446	1456	vibration of C–N group –CH_2 (symmetric bending)
1240	1236	1236	C–N stretching and N–H deformation
1030	1031	1032	–OH groups, C–O stretching

While examining the N–H stretching region at 3300 – 3276 cm^{-1}, there is a clear observable pattern whereby the porcine gelatine consistently depicts the highest wavenumber (3300 cm^{-1} in literature data, while 3276 cm^{-1} by experimental results). This systematic shift in peak position can be attributed to differences in the strength and distribution of hydrogen bonds within the protein structure [33]. The hydroxyl and amine groups in porcine gelatine appear to form slightly different hydrogen bonding networks compared to the halal sources of bovine and fish, resulting in these characteristic spectral shifts.

One particularly notable marker region is the symmetric $–CH_2$ bending vibration, occurring around 1456 – 1442 cm^{-1}. The experimental data reveal that the porcine gelatine exhibits a higher wavenumber of 1456 cm-1 compared to bovine gelatine at 1446 cm^{-1} and fish gelatine at 1450 cm^{-1}. This shift suggests the differences in the molecular packing and the side chain arrangements between the halal and non-halal gelatine sources based on the type of protein structure. Additionally, the C–N stretching and the N–H deformation bands around 1240 – 1231 cm^{-1} demonstrate subtle but consistent differences among the sources, with porcine gelatine typically exhibiting intermediate values between bovine and fish sources. So, when considered collectively, these spectral features can create a unique "fingerprint" that can help and guide the authentication of halal gelatine sources, especially in food and pharmaceutical products.

The discriminant analysis

The DA is a powerful MVDA technique widely used to classify observations into predefined groups based on their measured characteristics [34]. This method is particularly valuable when the objective is to uncover patterns in complex datasets, whereby multiple variables interact simultaneously to influence the group membership. Thus, by constructing a discriminant function that maximizes the separation between groups, the DA provides insights into the most critical variables contributing to classification, enabling more informed decision-making. Although DA was performed to evaluate the classification potential among porcine, bovine and fish gelatine, it is important to note that only a single reference sample was used per source. As such, the resulting model serves primarily as a proof-of-concept, highlighting distinguishing features rather than establishing a fully validated classification system. The limited sample size may lead to model overfitting. Therefore, the results should be interpreted cautiously. Future work will focus on expanding the dataset to include multiple batches and commercial samples to improve robustness and generalisation.

The study's DA results provide compelling evidence for establishing a reliable, scientifically based halal verification protocol using FTIR-ATR spectroscopy. The exceptional discrimination achieved in the 2000 – 1501 cm^{-1} region, with 100% training accuracy and high cross-validation success rates (83.33% for mammalian and 100% for marine sources), suggests that this spectral range should be the primary focus for halal verification methods. This region encompasses the crucial Amide I and II bands, which provide definitive information about protein secondary structure and molecular arrangements specific to different species. The statistical robustness of this region, demonstrated by an $F_{observed}$ value of 427 ($p<0.01$), indicates that it could serve as a reliable primary marker for halal certification processes. To enhance verification accuracy, a multi-region approach should be implemented. The complementary discrimination power observed in the 3500 – 3001 cm^{-1} region, which achieved 80% accuracy for marine sources and 100% accuracy for the training data, suggests that combining analyses from multiple spectral regions could provide a more comprehensive authentication. This dual-region approach would be particularly valuable for regulatory bodies and halal certification authorities, as it offers redundancy in verification and reduces the likelihood of false positives. The extremely low p-values ($p<0.01$) across these regions provide strong statistical support for implementing such a protocol in official halal certification processes.

However, the practical implications for industry implementation must be carefully considered. The varying cross-validation results across different spectral regions highlight the need for standardized sample preparation and analysis procedures. For instance, the lower accuracy in the 1000 – 650 cm^{-1} region suggests that protocols should include specific sample handling and data processing guidelines to minimize environmental and procedural variations. Furthermore, developing a standardized database of reference spectra from verified halal sources would be crucial for making reliable comparisons. This method's non-destructive nature, rapid analysis time, and relatively low cost make it particularly attractive for routine industrial quality control and regulatory compliance monitoring. Nevertheless, it should be complemented with other analytical techniques for definitive certification purposes.

The principal component analysis

The PCA is known for its widely used dimensionality reduction technique in MVDA, which transforms high-dimensional data into a lower-dimensional space while retaining as much variability as possible. By identifying new, uncorrelated variables called Principal Components (PCs), the PCA simplifies complex datasets, making it easier to visualize patterns, detect relationships and interpret underlying structures. Furthermore, the ability of PCA to capture maximum variance through a minimal number of components is explored, along with its implications for reducing redundancy in variables and enhancing interpretability [35].

Table 5. Classification matrix of training and cross-validation of discriminant analysis

Discriminating Model at Certain Wavenumber (cm^{-1})	Wilk's Lambda Test				Dataset	% Class
	$F_{observed}$	$F_{critical}$	p-value	α		
1000 – 650	6955	99	0.00014	0.01	**Training**	
					Mammal	100
					Marine	100
					Cross-Validation	
					Mammal	66.7
					Marine	0
1500 – 1001	8680	5859	0.00822	0.01	**Training**	
					Mammal	100
					Marine	100
					Cross-Validation	
					Mammal	100
					Marine	0
2000 – 1501	427	99	0.00233	0.01	**Training**	
					Mammal	100
					Marine	100
					Cross-Validation	
					Mammal	83.3
					Marine	100
2500 – 2001	11964751	5859	0.00022	0.01	**Training**	
					Mammal	100
					Marine	100
					Cross-Validation	
					Mammal	100
					Marine	0
3000 – 2501	403	99	0.00248	0.01	**Training**	
					Mammal	100
					Marine	100
					Cross-Validation	
					Mammal	33.3
					Marine	0
3500 – 3001	9430	99	0.00011	0.01	**Training**	
					Mammal	100
					Marine	100
					Cross-Validation	
					Mammal	80
					Marine	100
4000 – 3501	9334	99	0.00011	0.01	**Training**	
					Mammal	100
					Marine	100
					Cross-Validation	
					Mammal	100
					Marine	0

Table 6. The KMO test for all bovine, fish and porcine FTIR of 728 datasets generated by the XLSTAT software

Wavenumber (cm^{-1})	KMO Result
1000 – 650	0.774
1500 – 1001	0.756
2000 – 1501	0.539
2500 – 2001	0.782
3000 – 2501	0.624
3500 – 3001	0.784
4000 – 3501	0.695

Note: KMO value table: 0 – 0.49 (unacceptable); 0.50 – 0.59 (miserable); 0.60 – 0.69 (mediocre); 0.70 – 0.79 (middling); 0.80 – 0.89 (meritorious); 0.90 – 1.0 (excellent)

The KMO test results for all the fish, bovine, and porcine gelatines in the FTIR-ATR spectral regions, as depicted in **Table 5**, reveal varying degrees of sampling adequacy across the different wavenumber ranges from 4000 – 650 cm^{-1}, respectively. The KMO values range from 0.539 – 0.784, whereby the values above 0.70 are generally considered adequate for factor analysis [36-37]. Three spectral regions demonstrated good sampling adequacy: for 3500 – 30001 cm^{-1} range (0.784), 2500 – 2001 cm^{-1} range (0.782), and 1000 – 650 cm^{-1} range (0.774). Meanwhile, the mid-range region of 1500 – 1001 cm^{-1} reveals acceptable adequacy at 0.756, but the regions of 4000 – 3501 cm^{-1} (0.695), 3000 – 2501 cm^{-1} (0.624), and particularly 2000 – 1501 cm^{-1} (0.539) exhibit mediocre to poor sampling adequacy, suggesting that these spectral ranges may be less suitable for factor analysis or may require additional sampling to improve their reliability for the MVDA [38].

The PCA was conducted on a total of 728 datasets derived from fish, bovine, and porcine gelatine samples, focusing on their transformation across various FTIR-ATR wavenumber ranges of 4000 – 650 cm^{-1} regions, respectively. These regions were selected to represent the characteristic spectral bands for the different molecular vibrations, which are essential for distinguishing between all the gelatines types from these sources, as illustrated in Figure 6, which presents the PCA results through a biplot, providing a clear visual exploration of the gelatine dataset for fish, bovine and porcine, respectively. The PCs account for 98.71% to 99.99% of the variance within the FTIR-ATR spectral wavenumber range of 4000 – 650 cm^{-1}. In particular, the biplot reveals that bovine gelatine (grouped in blue circles) and fish gelatine (grouped in green circles) are distinctly separated and demonstrate no correlation with the porcine gelatine (grouped in red circles) across the FTIR-ATR spectral range. Interestingly, the PCA biplot confirms the distinct spectral profiles of fish, bovine, and porcine gelatine, demonstrating the capability of PCA as a robust analytical tool for halal verification. By effectively differentiating gelatine sources based on their FTIR-ATR spectral characteristics, the PCA facilitates the identification of porcine-derived gelatine, which is critical for ensuring compliance with halal standards.

Correlation of DA and PCA for preliminary halal authentication in gelatine

The correlation between the PCA and DA has emerged as a powerful chemometric strategy in the preliminary authentication of gelatine for halal purposes, particularly when analyzing spectral data derived from techniques of FTIR-ATR spectroscopy. Moreover, PCA, which an unsupervised multivariate technique, plays a important role in data exploration by reducing dimensionality and capturing the variance structure within complex spectral datasets. This process aids in visualizing clustering patterns based on the origin of gelatine typically porcine agains bovine sources. On the other hand, DA is a supervised classification method that leverages the input from PCA to maximize the separation among known categories, thus enabling precise classification and discrimination of gelatine sources.

Several latest studies have demonstrated the complementary role of PCA and DA in enhancing the reliability of halal authentication protocols. For instances, Salamah and co-workers employed PCA to assess the spectral variance among gelatine samples in the soft candy and then utilized DA to classify porcine and bovine gelatines with high sensitivity and specificity. The PCA score plots revealed distinct clusters, which were further validated by the DA model, achieving classification accuracy of 100% [39]. Meanwhile, Badrul and co-workers reinforced the findings by also applying FTIR spectroscopy with PCA for rapid detection of porcine gelatine in hard shell capsules on supplements products, respectively. The result highlighted the PCA provided the insights into group separation of bovine, porcine and mixture of both gelatines [40]. The synergy between PCA and DA not only improvise the interpretability and predictive capacity of analytical

models but also aligns with the growing demand for non-destructive, rapid and cost-effective methods in halal verification. Sani and co-workers illustrated how this integrated approach could detect authentication and quantification in food matrices, highlighting its broader applicability across various halal-sensitive products [41].

All above example underscore that PCA facilitates initial data structuring and variable reduction, which is critical when working with high-dimensional spectral datasets. while DA provided a definitive classification mechanism, therefore offering a statistically rigorous framework for halal authentication. From a practical standpoint, this correlation can serve as a decision-support tool for halal certifying bodies and industries seeking to comply with Islamic dietary laws. The combined use of PCA and DA ensures that subtle spectral differences are captured and utilized for reliable authentication. This methodological framework enhances traceability and transparency in gelatine sourcing, thereby strengthening consumer confidence in halal-labelled products. As advacements in spectroscopic instrumentation and computational tools continue, the application of such multivariate techniques is likely expand, contributing significantly to the standardize and scientific rigour of halal authentication systems.

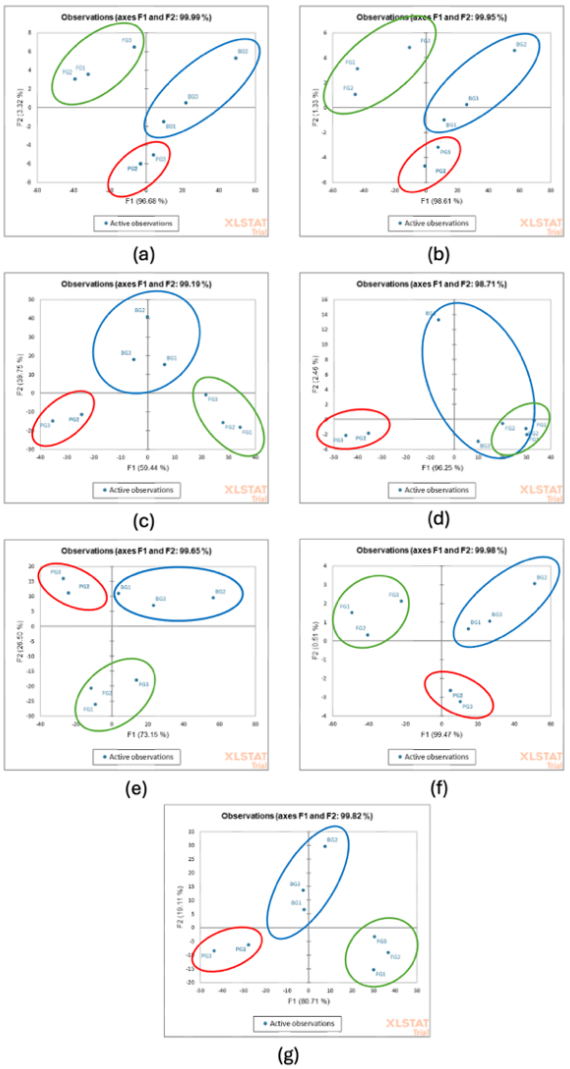

Figure 5. The PCA analysis of total 728 datasets transformation of fish, bovine and porcine with the observations value ranging from 98.71 – 99.99% of FTIR wavenumber of (a) 1000 – 650 cm^{-1}; (b) 1500 – 1001 cm^{-1}; (c) 2000 – 1501 cm^{-1}; (d) 2500 – 2001 cm^{-1}; (e) 3000 – 2501 cm^{-1}; (f) 3500 – 3001 cm^{-1}; (g) 4000 – 3501 cm^{-1}

Conclusions

This study highlights the robust and meaningful capability of the FTIR-ATR spectroscopy combined with DA and PCA for halal verification from the standard animal gelatine of fish, bovine and porcine, respectively. FTIR-ATR spectroscopy provides distinct spectral fingerprints, enabling the precise identification of all gelatine sources from wavenumbers 4000 – 650 cm^{-1}. Moreover, when combined with MVDA of DA and PCA, the method enhances classification accuracy and discrimination of halal and non-halal gelatine. Integrating pattern recognition through PCA also enhances the reliability of this approach, providing a powerful, rapid, and non-destructive tool for ensuring halal compliance in the food and pharmaceutical industries. Nevertheless, the preliminary findings suggest that differentiation of gelatine sources is feasible using the selected analytical approach. However, further validation with a larger and more diverse sample set is essential to ensure the reliability and applicability of the model in real-world halal authentication scenarios.

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