# Quantitative and Qualitative Halal Authentication Analysis of Malaysian Animal-Based Fermented Food Products Using HPLC-DAD, FTIR-ATR Spectroscopy and Chemometric Approaches

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***Abstract:*** *Halal status verification of animal-based fermented food products is critical for protecting consumer health and ensuring regulatory compliance in Muslim-majority markets. This study presents a comprehensive evaluation of qualitative and quantitative aspects of Malaysian fermented food products using integrated chemical, spectroscopic, microbiological, and chemometric approaches. Histamine quantification by HPLC-DAD revealed concentrations ranging from 44.2–570.3 mg/kg, with 47% of samples exceeding the Codex Alimentarius limit of 100 mg/kg, indicating potential chemical safety concerns. FTIR-ATR spectral profiling distinguished fermentation-induced lipid modifications and differentiated halal products from non-halal reference lard samples, revealing distinct molecular fingerprints across animal, plant, and fermented food matrices. Chemometric analysis using principal component analysis (PCA) and orthogonal partial least squares-discriminant analysis (OPLS-DA) in the wavenumber fingerprint region (1500–650 $cm^{-1}$) achieved clear sample discrimination and confirmed the absence of non-halal lipid adulteration. Microbiological assessments revealed total plate counts within acceptable regulatory limits, no detection of* Escherichia coli*, and controlled levels of* Staphylococcus aureus*, indicating satisfactory hygienic quality. These amalgamated findings establish a robust, multi-modal framework for halal authentication and quality control of fermented food products with direct implications for strengthening regulatory oversight, preventing food adulteration, and ensuring consumer protection in halal-certified food systems.*

***Keywords:*** *chemometric; fermented food; food safety; FTIR-ATR spectroscopy; halal authentication; histamine*

## ■ INTRODUCTION

In the Malaysian halal food sector, the halal concept represents a model that extends beyond mere permissibility to encompass wholesomeness, safety, nutritional integrity, and quality assurance. This integrated framework has gained prominence in response to globalized supply chains, heightened Muslim consumer awareness, and the evolving regulatory landscape mandated by Malaysia's MS1500:2019 standard, which formally incorporates safety requirements into halal food processing guidelines [1]. The mandate of the halal concept reflects Islamic principles of food consciousness, in which consuming food is not only a matter of legality but also of health and moral responsibility, resonating with both the Quran

and the philosophy of ensuring products are safe, authentic, and suitable for Muslim consumers. Traditional Malaysian cuisines prominently feature animal-based fermented foods such as *pekasam* (fermented freshwater fish) and *belacan* (shrimp paste), which are culturally significant and economically important. These fermented food products have been integral to Southeast Asian culinary heritage for centuries, contributing to consumer acceptance through their unique flavors, textures, and preservation properties [2]. However, despite their cultural values, producing these foods through unregulated or spontaneous microbial fermentation poses substantial challenges to achieving halal compliance. Inconsistencies in chemical composition, hygiene standards, fermentation timeframes, and microbiological management can compromise both the safety and quality attributes essential to halal principles. Recent studies have documented significant variations in the physicochemical and microbiological profiles of commercial *pekasam* available in Malaysian markets, highlighting the pressing need for systematic quality assessment and control methodologies [3].

Among the most significant chemical hazards in fermented food products derived from animal based is the formation of biogenic amines, particularly histamine, which forms through microbial decarboxylation of the amino acid, histidine (Fig. 1). These biogenic amines are low molecular-weight decarboxylation products formed during microbial fermentation and their accumulation in fermented food products can reach levels far exceeding documented toxic threshold [4]. Histamine, the primary biogenic amine of concern in fermented seafood, is produced when histamine-producing bacteria, particularly certain species of *Bacillus*, *Clostridium*, and proteolytic *Vibrio* species, proliferate under improper storage and fermentation conditions and catalyze the decarboxylation reaction [5]. Increasing amount of histamine levels violates the safety criteria of safety and wholesomeness by inducing scombroid poisoning, urticaria, headache, diarrhea, and in severe cases, bronchospasm or cardiac arrhythmias, particularly in immunocompromised individuals or those on certain medications such as monoamine oxidase inhibitors, respectively [6].

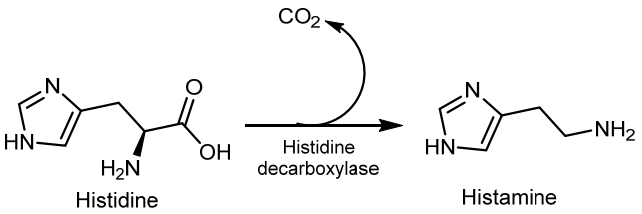


**Fig 1.** Histidine decarboxylation to histamine by histidine decarboxylase is a key reaction in the formation of histamine in fermented food products

Studies from Malaysian fermented seafood markets have documented histamine levels in *belacan* samples ranging from undetectable to 143 mg/kg, with approximately 47% of northern Malaysian samples exceeding the recommended 50 mg/kg safety threshold established by the USA Food and Drug Administration (FDA) and over 26% surpassing 500 ppm [7]. Additionally, Malaysian *pekasam* has shown histamine concentrations of 1694 mg/kg, alongside substantial accumulation of other biogenic amines, such as putrescine and cadaverine, indicating severe microbial protein degradation. The European Commission (EC) regulation 2073/2005 establishes a limit of 100 mg/kg with a maximum of 200 mg/kg for individual samples of fermented fish products, while the FDA adopts the more restrictive 50 mg/kg defect action level [8]. These regulatory frameworks underscore the global recognition of histamine as a critical food safety hazard, yet traditional Malaysian fermented food products often exceed these internationally recognized limits, posing a genuine threat to consumer health and market access.

Modern analytical technologies offer powerful solutions to systematically address this complex chemical in Malaysian fermented food products. The Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) provides a rapid, non-destructive methodology for chemical fingerprinting of complex food matrices. The technique captures vibrational spectra across the infrared region, enabling detection of functional group-specific vibrations associated with lipids, carbohydrates, proteins, and other biochemical constituents. This spectroscopic approach facilitates the detection of minute molecular changes associated with protein degradation, lipid oxidation, and spoilage

processes, which are indicative of product quality and safety [9]. When coupled with chemometric methods such as principal component analysis (PCA), partial least squares regression (PLSR), and hierarchical clustering, the FTIR-ATR transforms raw spectral data into actionable qualitative and quantitative information. Chemometrics enables the classification of samples, the detection of adulteration, the prediction of quality attributes, and the verification of halal compliance through pattern recognition and multivariate statistical modelling [10]. FTIR-ATR spectroscopy combined with multivariate data analysis has already demonstrated considerable potential for halal assurance applications, including the identification of meat adulteration in processed foods, the differentiation of animal fats and non-halal lipid sources, and the verification of food provenance and authenticity.

Herein, this study addresses a significant research gap: most existing investigations focus on isolated parameters, such as histamine levels or microbial enumeration, whereas this work simultaneously evaluates chemical, microbiological, and spectroscopic dimensions through advanced analytical chemistry and chemometric analysis. Such a comprehensive perspective is rarely applied to regional fermented food products, yet it is crucial for ensuring consumer protection and regulatory compliance for these products. Furthermore, the spectroscopic-chemometric platform provides quantitative and qualitative analysis, offering a rapid, cost-effective alternative to conventional laboratory methods and facilitating real-time quality monitoring and process optimization. Ultimately, this research also advances the integration of contemporary analytical techniques with halal principles, enhancing scientific knowledge while promoting the broader goal of ensuring that culturally significant, traditionally produced fermented foods from Malaysia remain safe, authentic, and acceptable to Muslim consumers in domestic and international markets.

## ■ EXPERIMENTAL SECTION

### Materials

The fermented food samples were purchased from different states in Malaysia, including *belacan* from Kedah and Melaka, *budu* from Kelantan and Terengganu, *pekasam* from Kedah, and squid and prawn *cincalok* from Melaka. Animal meats of chicken, beef, mutton, anchovies, *ikan tongkol* (*Euthynnuss* sp.), *ikan selar* (*Decapterus* sp.), squid (*Decapodiformes* sp.), and prawn (*Dendrobranchiata* sp.) were obtained from a local supermarket in Muar, Johor, Malaysia. Histamine (HPLC grade) (Supelco, USA), perchloric acid (70%, Sigma-Aldrich, USA), dansyl chloride (Sigma-Aldrich, USA), NaOH pellets (Merck, Germany), $NaHCO_3$ (Bendosen, Norway), ammonia (Bendosen, Norway), acetone (Bendosen, Norway), acetonitrile (HPLC grade) (Sigma-Aldrich, USA), and ammonium acetate (Systerm Chemicals, Malaysia) were used in this work. Ultrapure water was obtained from Milipore purification system (Elix®/Milli-Q®, USA), palm oil standard (Sigma-Aldrich, USA), lard standard (Sigma-Aldrich, USA), fish oil from menhaden standard (Sigma-Aldrich, USA), corn oil standard (Sigma-Aldrich, USA), petroleum ether (boiling range: 40–60 °C) (R&M Chemicals. UK), $MgSO_4$ (Systerm Chemicals, Malaysia), ethanol (R&M Chemicals, UK), plate count agar (Merck, Germany), mineral-modified glutamate agar (HiMedia, India), tryptone-bile-X-glucuronide agar (HiMedia, India), and Baird-Parker agar (HiMedia, India) were used in this work. All chemicals were used as received without further purification.

### Instrumentation

The HPLC system used was Agilent 1290 Infinity II series (Agilent Technologies, USA) equipped with an autosampler and a Diode Array Detector (DAD). The column was Zorbax Eclipse XDB C18 (4.6 × 100 mm, 3.5 µm). The column was equilibrated at 40 °C and 1.0 mL/min. 0.1 M of ammonium acetate at pH 7.9 was used as solvent A, and solvent B was acetonitrile. The elution gradient is reported in Table 1. The system was

**Table 1.** The elution gradient

| Time (min) | A (%) | B (%) |
|---|---|---|
| 0 | 65 | 35 |
| 15 | 30 | 70 |
| 25 | 5 | 95 |
| 30 | 5 | 95 |
| 31 | 65 | 35 |

equilibrated for 5 min before the next run. The injection volume for standard materials and samples was 20 µL; histamine was detected at 254 nm.

The FTIR spectra were obtained using a Nicolet iS5 FTIR spectrometer (Thermo Scientific, USA) with a 4 $cm^{-1}$ resolution and 32 scans per spectrum (4000–650 $cm^{-1}$). An ATR cell analyzed 0.5 mg of the extracted oil samples and standards with a blank diamond ATR scan for reference. Spectra were processed using OMNIC™ software (v8.2) with ATR correction, baseline correction, and smoothing before being saved as a CSV file.

## Procedure

### *Sample preparation*

Animal meats of chicken, beef, mutton, anchovies, *ikan tongkol*, *ikan selar*, squid, and prawn were prepared according to Nazri et al. [11] with minor modification. The fermented food products of *budu*, *belacan*, *pekasam*, and *cincalok* were dried in a drying oven (Memmert, Germany) at 60 °C for 2–3 days. All dried samples of animal meats and fermented food products were then ground using a commercial grinder (Sharp, Japan), collected, and kept in a re-sealable bag at −20 °C for further analysis.

### *Samples and standard extraction*

About 5 g of dried histamine samples, animal meats, and fermented food products were added to 20 mL of 0.4 M perchloric acid and homogenized with an Ultra-Turrax homogenizer in an ice bath. The mixture was then centrifuged using a centrifuge (Kubota, Japan) at 7,000 rpm for 10 min at 4 °C. The supernatants were collected, and the final volume was adjusted to 25 mL with perchloric acid extraction solution.

### *Derivatization of standard histamine and sample extraction*

The derivatization of histamine and sample extraction based on the method established by Altieri et al. [12] with minor modification. Approximately 0.5 mL of histamine and extracted samples were mixed with 100 µL of 2 M NaOH and 150 µL saturated $NaHCO_3$. Then, 1 mL of 10 mg/mL dansyl chloride solution in acetone was added. The histamine and extracted samples were incubated at 40 °C for 45 min, then cooled to room temperature for 10 min. The residual dansyl chloride was removed by adding 100 µL of 1 M ammonia solution. After 30 min at room temperature. The histamine and extracted samples were adjusted to 5 mL with acetonitrile and filtered through a 0.45 µm syringe filter.

### *Extraction of oils*

Approximately 20 g of dried and ground animal meat and fermented food samples were placed in a 30 × 100 mm extraction thimble made of thick filter paper and inserted into the primary chamber of the Soxhlet extractor (Electrothermal, USA). Then, 200 mL of petroleum ether was used as the solvent and heated to reflux for 6 h. Post-extraction, a spoonful of $MgSO_4$ was added, and the mixture was filtered through filter paper (Whatman, UK). The filtrate was then concentrated using a rotatory evaporator (EYELA, Japan). Afterward, the oils were collected and analyzed by FTIR-ATR.

### *Standard calibration curve*

A stock solution of histamine was prepared at a concentration of 3.2 mg/mL by dissolving it in ultrapure water. Five standard materials for the calibration curve (in the range 30–700 mg/kg) were prepared daily, adding each histamine aqueous solution to 5 g of blank matrix.

### *Chemometric analysis*

The full FTIR-ATR spectra were extracted from their transmittance values to generate a comprehensive PCA dataset in a .csv file. The chemometric analysis was performed using Streamlit software (v1.51.0) through established Python code, which enables the interactive visualization and processing of FTIR-ATR spectral data, including PCA and orthogonal partial least square discriminant analysis (OPLS-DA) [13]. Notably, PCA and OPLS-DA were used to reduce dimensionality and for classification, highlighting variations in the FTIR-ATR spectra at specific wavenumbers [10]. For PCA of FTIR-ATR spectra, the wavenumber range of 1500–650 $cm^{-1}$ was selected, as it contains highly diagnostic vibrational features that are particularly sensitive to structural differences among fats, oils, and other organic matrices. These spectral regions were chosen for their high potential to capture key molecular vibrations

arising from bending, rocking, and skeletal motions that are not easily observed at higher wavenumbers [14]. The number of principal components (PCs) was optimized to obtain optimum differentiation among samples. The differentiation result of the samples was observed using a PCA score plot.

### *Quantitative microbiological analysis*

All microbiological analyses were performed according to the British Standard ISO (BS ISO 4833: 2022; BS ISO 16649-1: 2018; BS ISO 6888-1: 2021) [15-16]. For enumeration, 10 g of each fermented food sample was aseptically taken and homogenized for 2 min with a vortex in 90 mL of sterilized peptone water. Homogenized samples were serially diluted 10-fold in 9 mL of sterile peptone-buffered water to $10^{-7}$. Then, 1 mL of sample from each dilution was pour-plated in duplicate onto plate count agar for total plate count (TPC) before incubation at 30 °C for 72 h. For *Escherichia coli* and *Staphylococcus aureus* enumeration, 0.1 mL of serially diluted samples was spread-plated onto mineral-modified glutamate agar before transferring into selective Tryptone-Bile-X-Glucuronide (TBX) agar and Baird-Parker agar supplemented with egg yolk tellurite emulsion (50 mL $L^{-1}$), respectively. Both procedures involving *E. coli* and *S. aureus* enumeration were incubated at 44 °C for 24 h and 37 °C for 48 h, respectively. Typical glucuronidase-positive *E. coli* appeared to be blue-greenish colonies, while typical positive-coagulase *S. aureus* were black-shiny colonies. *E. coli* ATCC 11775 and *S. aureus* ATCC 6538 were used as positive controls. Microbial colonies were enumerated as colony-forming units per mL (CFU $mL^{-1}$).

## ■ RESULTS AND DISCUSSION

### Histamine Analysis by HPLC-DAD

The HPLC-DAD quantification produced a calibration function of Y = 1.6645x + 80.6189 with $R^2$ = 0.993, indicating acceptable linearity and detector response across the working range of histamine (30–700 mg/kg) for the present study, as tabulated in Table 2. The high $R^2$ supports reliable interpolation of peak areas to concentration, although the non-zero intercept of 80.6189 indicates a systematic baseline offset that should be considered when quantifying low-concentration samples and when estimating limits of detection (LOD) and quantification (LOQ), respectively. The HPLC-DAD remains a robust approach for histamine analysis because of its reproducibility and spectral confirmation capabilities, but method validation must explicitly address matrix effects, extraction recoveries, and derivatization efficiency, issues emphasized in recent method reviews for biogenic amines in complex food matrices [17]. However, such methodological robustness is essential when analytical data are used not only for safety assessment but also to support halal claims, where accuracy, transparency, and scientific defensibility are essential components of halal assurance systems [18].

**Table 2.** The histamine concentration in animal adipose tissue and animal-based Malaysian fermented food products

| Sample | Area | Amount (mg/kg) |
|---|---|---|
| Mutton | n.d | n.d |
| Beef | 528.6 ± 2.3 | 269.1 ± 1.4 |
| Chicken | n.d | n.d |
| *Decapterus* sp. | 783.6 ± 2.5 | 422.3 ± 1.5 |
| *Euthynnuss* sp. | 154.3 ± 1.6 | 44.2 ± 0.9 |
| Prawn | n.d | n.d |
| Squid | n.d | n.d |
| Anchovies | 411.9 ± 1.3 | 199.1 ± 0.8 |
| BD1 | n.d | n.d |
| BD2 | n.d | n.d |
| BD3 | n.d | n.d |
| BD4 | 859.4 ± 1.1 | 467.8 ± 0.6 |
| BL1 | 672.9 ± 0.4 | 355.8 ± 0.2 |
| BL2 | 1029.9 ± 0.6 | 570.3 ± 0.4 |
| BL3 | 893.5 ± 0.7 | 488.4 ± 0.4 |
| BL4 | 1000.7 ± 1.9 | 552.8 ± 1.1 |
| P1 | n.d | n.d |
| C1 | n.d | n.d |
| C2 | n.d | n.d |

Remark: All were analyzed in duplicate, and the reported values represent the mean of two independent injections to ensure analytical reliability and reproducibility. n.d = not detected

Chemically, histamine is formed by microbial decarboxylation of free histidine, catalyzed by histidine decarboxylase. This reaction is strongly influenced by intrinsic factors, such as amino acid composition, pH, and water activity, whereas extrinsic factors, such as

temperature, hygiene, and fermentation conditions, may also affect histamine formation [19]. The absence of detectable histamine in several animal adipose tissues (such as mutton, chicken, prawn, and squid) and some fermented foods of BD1, BD2, BD3, P1, C1, and C2 indicates minimal enzymatic decarboxylation, reflecting good raw materials quality and controlled processing conditions [16]. In contrast, detectable histamine levels in *Decapterus* sp., *Euthynnuss* sp., anchovies, and specific fermented food samples (BD4, BL1, BL2, BL3, and BL4) are chemically consistent with higher free histidine availability and favorable microenvironments for decarboxylase-positive bacteria, as widely reported in recent biogenic amine studies [20-21]. The representative HPLC-DAD chromatograms of the analyzed histamine standard, animal meat, and a sample of fermented food products are shown in Fig. 2.

In comparing regulatory frameworks for histamine content, Malaysia's current monitoring practice under the Malaysian Food Act 1983 and Food Regulations 1985 does not specify a formal statutory maximum for histamine in foods, but enforcement agencies often adopt

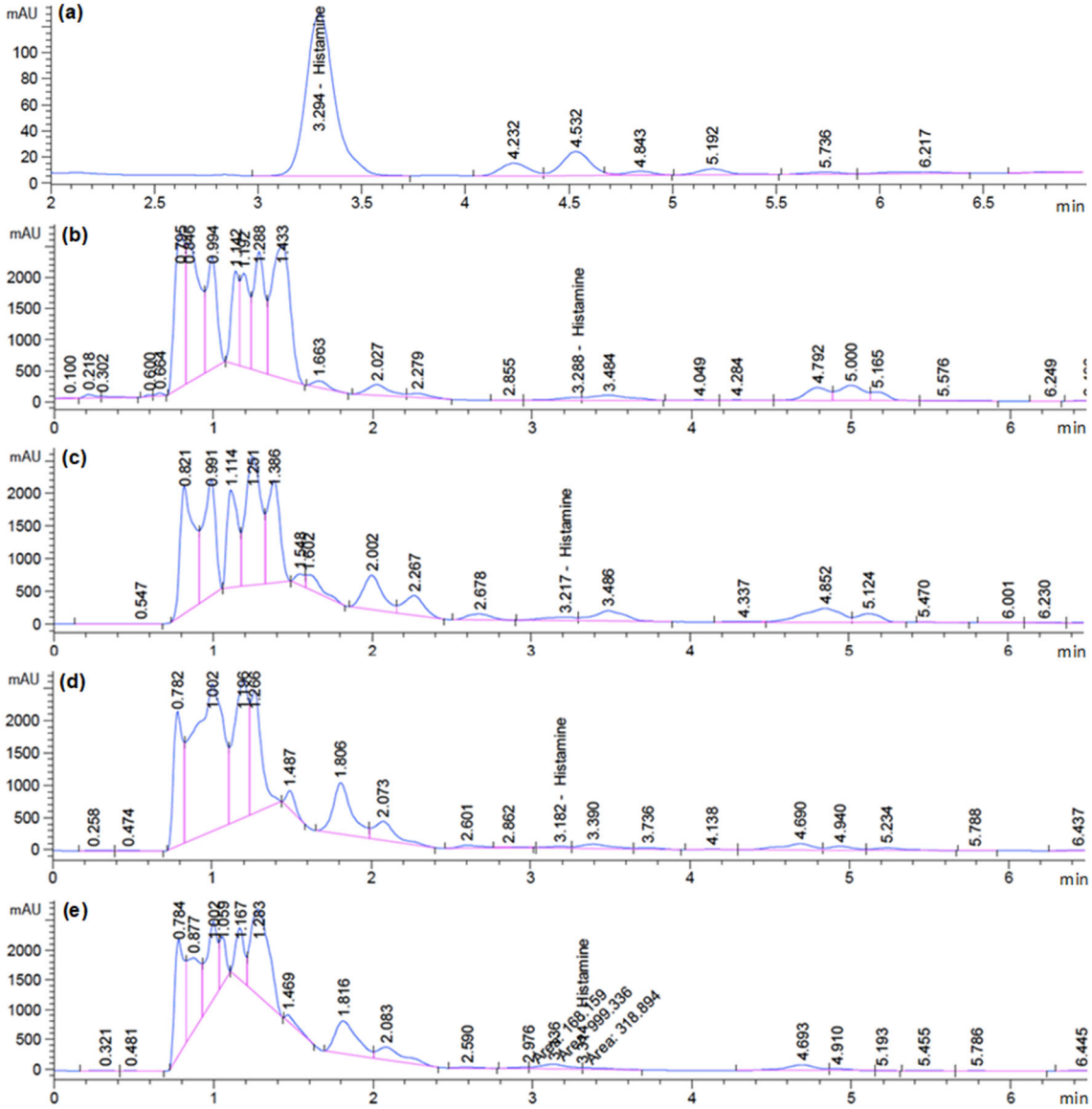


**Fig 2.** The representative HPLC-DAD chromatogram of (a) standard histamine at a concentration of 100 mg; (b) beef; (c) *Euthynnuss* sp.; (d) BD3, and (e) BL2

a 50 mg/kg (ppm) guideline for scombroid-forming fish and seafood as a conservative threshold for quality and safety screening [22]. This value aligns with the defective action levels commonly used in seafood safety surveillance and serves as a practical benchmark to protect consumers from spoilage-associated toxicological risks. Meanwhile, the Codex Alimentarius Commission, which sets internationally recognized food standards, recommends that the mean histamine concentration in susceptible fish species should not exceed 100 mg/kg, with no individual sample above 200 mg/kg and for certain fermented fish products such as fish sauce, a maximum of 400 mg/kg has been established to account for the inherently higher biogenic amine content from controlled fermentation [23]. These international limits reflect a risk-based approach that balances analytical feasibility and public health protection across diverse processing conditions and global markets.

Relating these limits to the halal concept underscores that halal assurance transcends mere permissibility to encompass wholesomeness, safety, and quality inherent in the safety dimension. From a chemical safety perspective, histamine is a biologically active amine whose elevated levels can provoke adverse reactions in susceptible individuals; therefore, maintaining histamine concentrations well below both the Malaysian monitoring threshold and the Codex Alimentarius limit is consistent with safety expectations for minimizing potential health hazards. Analytical data demonstrating histamine levels substantially below these benchmarks not only satisfy regulatory and international standards but also support halal integrity by evidencing that processing, storage, and handling practices effectively mitigate biochemical spoilage and microbial decarboxylation pathways that naturally generate histamine [24]. In essence, adherence to conservative histamine limits reinforces the scientific foundation of safety by ensuring that products are chemically safe and wholesome, thereby abiding by the broader objectives of halal food quality and consumer protection in both national and international contexts.

### FTIR-ATR Analysis

The FTIR spectroscopy coupled with ATR analysis of crude fat enables the detection of non-halal lipid adulteration and reveals early markers of lipid spoilage, such as increases in free fatty acids, oxidation bands, and structural disorder, which collectively reflect the safety, quality, and wholesomeness of the fermented products. Fig. 3 shows the FTIR spectra of oil from animal meat, marine, and vegetables, while Fig. 4 shows the FTIR spectra of the local fermented food sample, including *pekasam*, *budu*, *belacan,* and *cincalok*.

The spectra showed a weak cis C–H stretching band in the 3000 $cm^{-1}$ regions, along with strong aliphatic CH and $CH_2$ stretching vibrations at 2954, 2919, and 2849 $cm^{-1}$, which are characteristic of saturated and unsaturated fatty acids, and it appeared consistently in all samples (Fig. 2) [25]. Minor bands at 2354 and 2333 $cm^{-1}$, attributed to conjugated C=C species, were detected in several fermented samples (BD3, BD4, P1, C1) and some marine fats (SQD, PRN), likely reflecting secondary metabolites or early oxidative processes. These peaks were generally less prominent in vegetable oils and fresh animal fats. Although these features do not directly indicate adulteration, they may represent secondary metabolites associated with fermentation or early oxidative changes in the lipid fraction [25]. Despite overall spectral similarity in this region, even small variations in peak intensity and band shape are critical for identifying non-halal fat adulteration in complex fermented matrices. These subtle differences, particularly those distinguishing halal fats from lard, are often too slight to detect visually but can be effectively captured and interpreted through chemometric modelling.

The most distinctive spectral differences between fermented and unfermented fats were observed in the carbonyl region. Fresh animal fats and vegetable oils exhibited a strong ester carbonyl peak at 1744 $cm^{-1}$, indicating triglycerides, whereas this peak was noticeably weaker or less prominent in the fermented samples (Fig. 4) [26]. This reduction reflects the hydrolysis of triglycerides during fermentation, in which microbial or endogenous lipases cleave ester bonds, releasing free fatty acids. Consistent with this process, a clear carbonyl band at approximately 1707 $cm^{-1}$, corresponding to C=O stretching of free fatty acids (FFA),

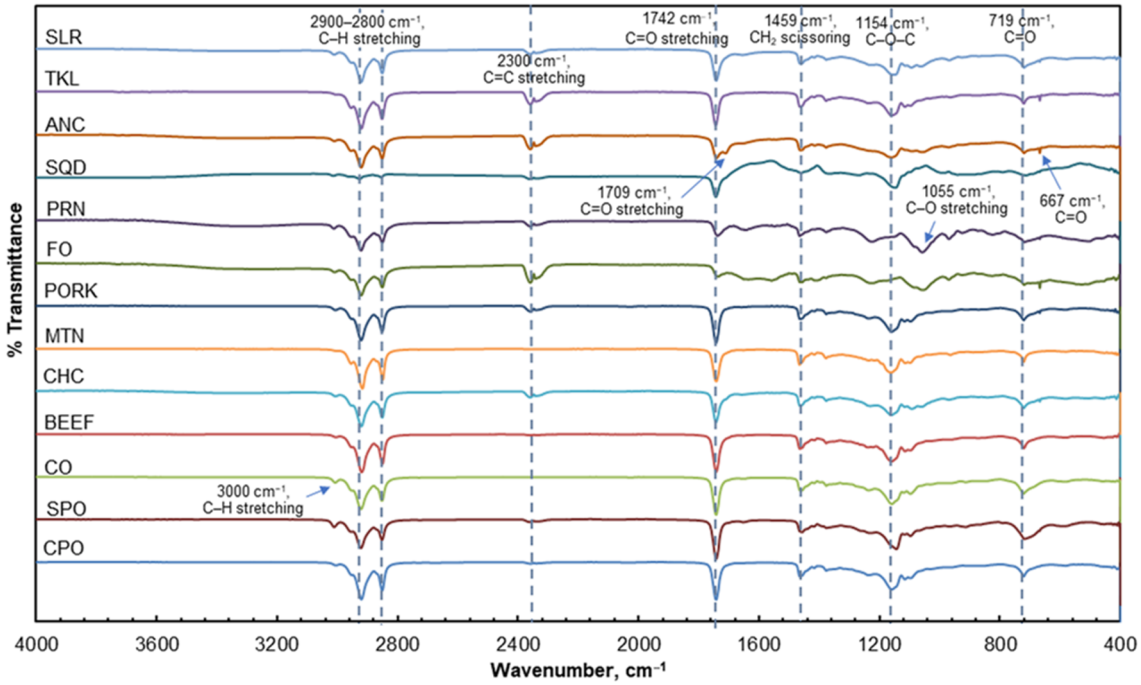


**Fig 3.** The FTIR spectra of fat from marine fish, including *Decapterus* sp. (SLR), *Euthynnuss* sp. (TKL), anchovies (ANC), squid (SQD), prawn (PRN), standard fish oil from menhaden (FO), land animals, including lard (PORK), mutton (MTN), chicken (CHC), cow (BEEF), and vegetable oils, including standard corn oil (CO), standard palm oil (SPO), commercial palm olein (CPO)

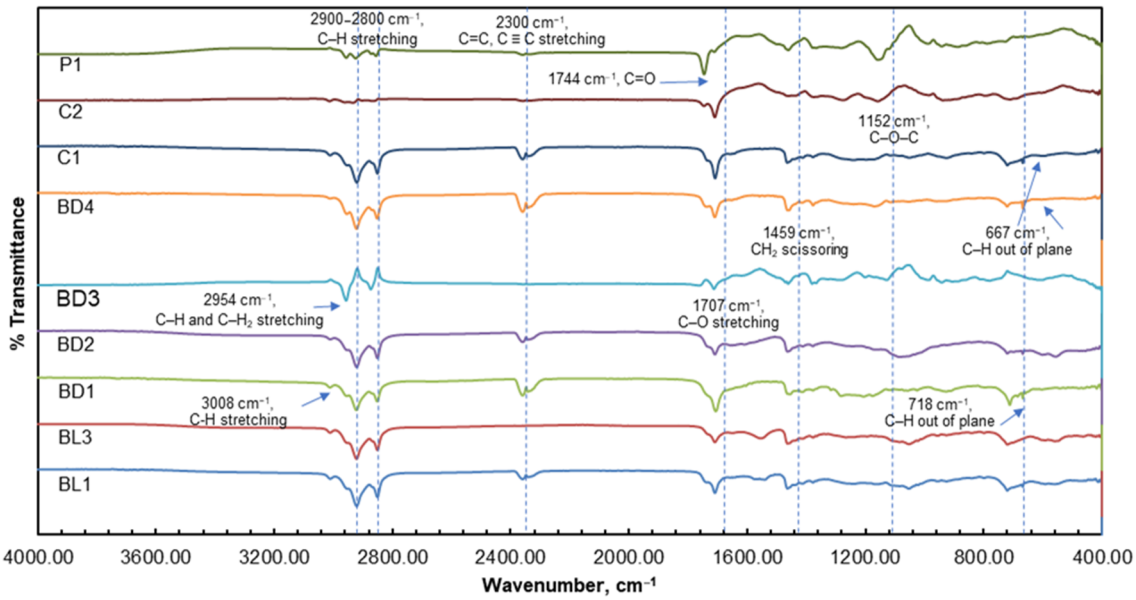


**Fig 4.** The FTIR spectra of *halal* fermented food products, including *pekasam* (P1), *cincalok* (C1 and C2), *budu* (BD1, BD2, BD3, and BD4), and *belacan* (BL1 and BL3)

was observed exclusively in fermented products, including anchovies, but was absent in fresh meat fats and refined vegetable oils. The simultaneous weakening of the 1744 $cm^{-1}$ band and emergence of the 1707 $cm^{-1}$ peak therefore provides strong evidence of fermentation-driven lipolysis. In pure fats, including lard, only the

ester carbonyl band is typically dominant; thus, chemometric modelling of this region helps distinguish non-halal fats even when blended with fermented matrices [27]. The band at 1459 $cm^{-1}$ corresponds to $CH_2$ bending vibrations of fatty acid chains, a feature commonly observed in both fermented fat extracts and reference fats. Although not directly linked to halal detection, this additional spectral feature further contributes to sample differentiation. The C–O–C stretching is observed at 1152 $cm^{-1}$, likely due to residual glycerol or polysaccharides co-extracted with the fat [28]. These features do not contribute to halal authentication but may introduce additional variance for chemometric classification. The peak at 1055 $cm^{-1}$ in the marine-derived crude fats of FO and PRN corresponds to C–O stretching vibrations associated with mono- and diglycerides, phospholipid components, and early oxidation products, which are more prominent in marine oils owing to their naturally higher phospholipid content and susceptibility to oxidative modification [29].

Looking at the fingerprint region, peaks at 718 $cm^{-1}$ ($CH_2$ rocking) and 667 $cm^{-1}$ (CH out-of-plane bending) were observed in several samples, including BD3, C1, and BD4 [30]. While these vibrations occur in both halal fats and lard, their relative intensities and shapes may differ slightly depending on fatty acid composition. These subtle differences are not reliable as standalone halal markers, but when included in multivariate analysis, they contribute to the broader spectral profile that supports halal and non-halal discrimination [31]. FTIR-ATR analysis of crude fat extracts from the fermented samples may also enable spoilage detection by monitoring changes in lipid functional groups [29]. Spoiled samples typically exhibit an increase in the free fatty acid carbonyl band (~1707 $cm^{-1}$), a reduction in ester carbonyl intensity (~1744 $cm^{-1}$), the appearance of oxidized carbonyl compounds (1710–1730 $cm^{-1}$), and changes in the aliphatic $CH_2$ stretching signal (2954–2849 $cm^{-1}$). Shifts in the fingerprint region (718 and 667 $cm^{-1}$) further indicate structural degradation of fatty acid chains. Combined with chemometric analysis, these spectral patterns enable classification of spoiled and acceptable fermented products solely from crude fat profiles. These findings demonstrate that FTIR-ATR analysis of crude fat extracts preserves the essential spectral markers required for detecting non-halal lipid adulteration while simultaneously providing indicators of lipid degradation, such as increased free fatty acids and early oxidation. Using chemometric tools, FTIR data enable robust classification and rapid evaluation of authenticity and fat quality in Malaysian fermented foods.

## PCA

The PCA serves as an essential exploratory tool for interpreting the high dimensionality of FTIR-ATR spectral data generated from Malaysian animal-based fermented food products by the oil extraction method, using the fingerprint region at 1500–650 $cm^{-1}$. By transforming the complex absorbance matrix into a set of orthogonal principal components, PCA reveals meaningful spectral variations associated with biochemical composition and product quality. This approach enables clear visualization of sample clustering, detection of subtle differences in functional group signatures, and identification of wavelength regions contributing most to variability. The PCA also accelerates decision-making by transforming large datasets, such as FTIR-ATR spectra, into easily visualized score plots, empowering researchers, regulatory authorities, and others to verify compliance more efficiently, especially for early detection of quality deterioration and for strengthening traceability frameworks [32].

Fig. 5 revealed clear spectral differentiation among the extracted oils from chicken, beef, mutton, anchovies, *Euthynnuss* sp., *Decapterus* sp., squid (*Decapodiformes* sp.), and prawn (*Dendrobranchiata* sp.) with standards of palm oil, lard, fish oil from menhaden, corn oil, and commercial palm oil, demonstrating the strong discriminative capability of FTIR-ATR fingerprint when subjecting to PCA modelling. The score plot showed that samples clustered by biological origin, indicating that compositional variation was captured by the principal components. Plant-derived fatty acids from CO, SPO, and CPO formed a compact, consistent cluster, highlighting their more uniform profiles and lower variability compared to animal-derived matrices. In contrast, the

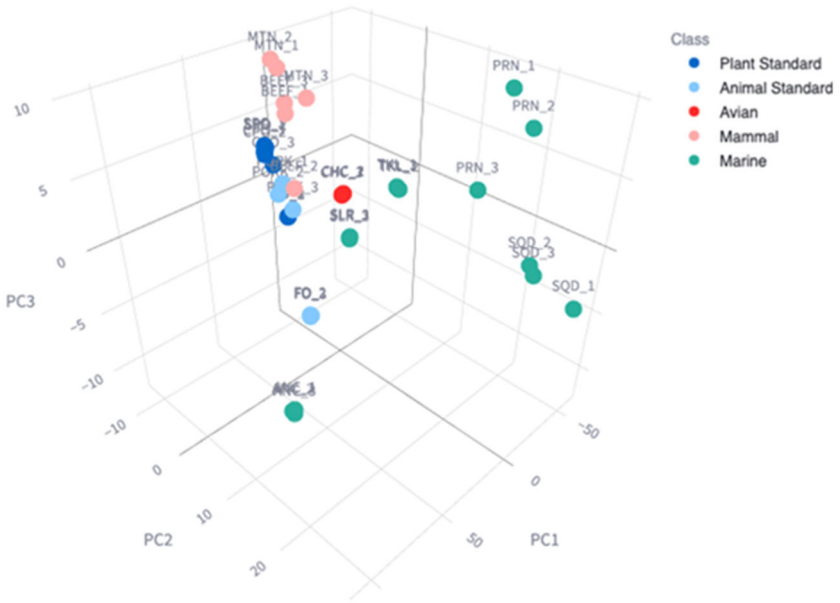


**Fig 5.** The 3D PCA interface created with the Streamlit software framework score plot showing clear separation of standard corn (CO), standard palm (SPO), commercial (CPO), chicken (CHC), beef, mutton (MTN), lard (PORK), prawn (PRN), squid (SQD), anchovies (ANC), *Euthynnuss* sp. (TKL), *Decapterus* sp. (SLR), and standard fish from menhaden (FO) oils samples based on FTIR-ATR fingerprint region, highlighting distinct clustering patterns by difference in fatty acid composition

animal-based samples exhibited wider dispersion, particularly among mammals (beef, mutton, and lard) and avian (chicken), suggesting greater heterogeneity in their fatty acid composition [33]. Meanwhile, marine-derived samples occupied a distinct region of score space, driven by the unique spectral contributions associated with long-chain polyunsaturated fatty acids commonly present in fish oils. Importantly, the lard samples aligned closely within the mammalian domain, reinforcing the model's ability to capture species-specific spectral markers relevant to halal authentication. The clear group separation across the first three latent variables provides robustness to the FTIR-ATR-chemometric approach for discriminating between lipid sources via oil extraction.

Fig. 6 demonstrates a coherent separation pattern that reflects the underlying chemical diversity of halal fermented food products and, importantly, supports the broader aims of halal authentication assessment. Each fermented food product of *budu* (BD1, BD2, BD3, and BD4), *belacan* (BL1, BL2, BL3, and BL4), *pekasam* (P1), and *cincalok* (C1 and C2) forms its own cluster, indicating consistent intra-product spectral characteristics and distinct inter-product differences. This stability suggests that their lipid profiles are naturally distinct yet internally reproducible, a key requirement for building reliable chemometric models in halal verification. Notably, lard, as a forbidden ingredient for Muslim, occupies a separate region of the PCA, as this clear spatial separation from all fermented food samples reinforces the ability of FTIR-ATR-PCA to discriminate halal matrices from non-halal sources (lard). Knowing lard typically exhibits unique absorption features associated with its fatty acid composition, its distance from the fermented food product clusters confirms that none of the analyzed products show spectral tendencies toward non-halal adulteration. From a halal standpoint, ensuring both permissibility and integrity is essential, especially when it comes to Muslim direct consumption of food products. These results have several important implications, as the distinct positioning of lard away from *budu*, *belacan*, *pekasam*, and *cincalok* demonstrates the robustness of PCA in detecting potential cross-contamination or adulteration. This supports science-based halal verification, especially for

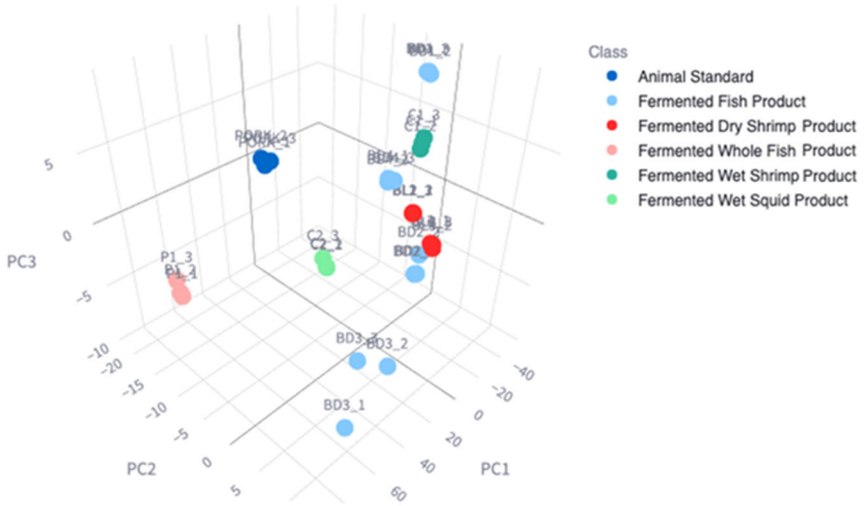


**Fig 6.** The PCA interface created with the Streamlit software framework score plot showing clear clustering of lard separated from *halal* fermented food products of *budu* (BD1, BD2, BD3 and BD4), *belacan* (BL1, BL2, BL3 and BL4), *pekasam* (P1) and *cincalok* (C1 and C2) for distinct discrimination based on respective FTIR-ATR spectral variations at fingerprint region

products produced in small or medium-scale artisanal settings. Moreover, the tight clustering of replicates within each fermented food product group shows chemical consistency, an indicator of stable production practices. This reproducibility aligns with safety principles involving hygiene, quality, and controlled processing [34]. PCA also serves as an early screening tool for quality control, flagging abnormal samples that deviate from their expected cluster. This contributes to preventive control in halal supply chains, ensuring that any irregularity due to adulteration, spoilage, or process deviation is rapidly detected.

## OPLS-DA

OPLS-DA has become an increasingly influential chemometric approach in halal research, offering a refined pathway for distinguishing permissible ingredients and detecting non-halal contaminants with high precision. As food matrices grow more complex, especially in traditional fermented products, processed foods, and multi-ingredient formulations, there is a growing need for analytical methods that not only identify differences but also explain them clearly. OPLS-DA fulfils this role by separating predictive variation directly linked to class differences, such as halal vs. non-halal, from orthogonal variation (unrelated noise), producing highly interpretable models that sharpen classification power [35].

The OPLS-DA model presented in Fig. 7 provides a well-structured separation of all sample classes, underscoring its effectiveness in resolving subtle chemical differences relevant to halal authentication. Plant and animal standards, avian, mammalian, and marine oils each occupy distinct regions of the score plot, demonstrating that the spectral variation related to their fatty acid composition is highly class-specific. This separation confirms that the OPLS-DA algorithm successfully isolates predictive variation differences truly associated with sample categories while filtering out unrelated orthogonal noise. The mammalian oils from beef, mutton, and lard form a compact cluster, distinctly separated from the avian and marine samples. This is especially important in halal authentication, as mammalian lipids often share overlapping characteristics with other animal fats, yet the model clearly distinguishes them. Such discrimination strengthens confidence that potential non-halal adulterants, such as lard, can be detected even in complex mixtures. However, marine-derived oils plot far from avian and mammalian groups, reflecting their unique lipid signatures and further

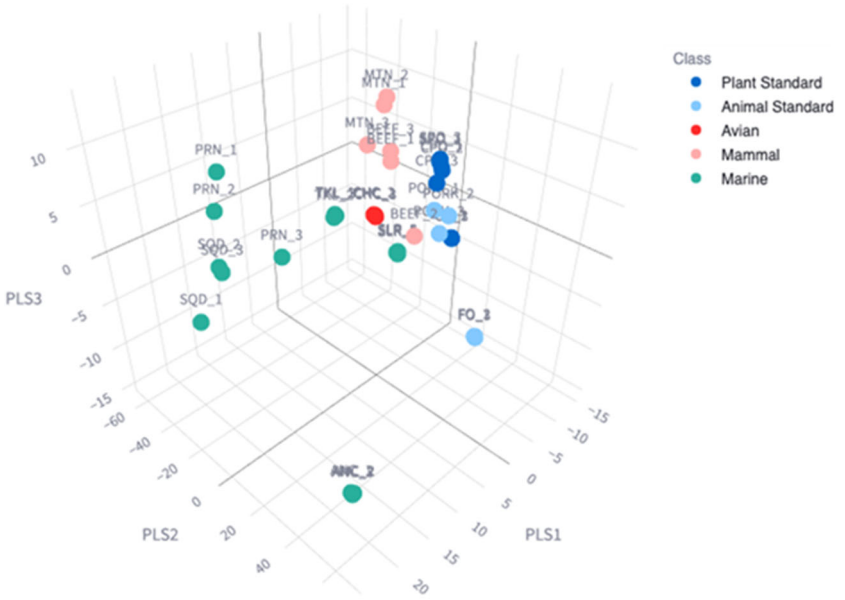


**Fig 7.** Three-dimensional OPLS-DA created with the Streamlit software framework score plot illustrating distinct separation among standard corn (CO), standard palm (SPO), commercial (CPO), chicken (CHC), beef, mutton (MTN), lard (PORK), prawn (PRN), squid (SQD), anchovies (ANC), *Euthynnuss* sp. (TKL), *Decapterus* sp. (SLR) and standard fish from menhaden (FO) oils samples based on their FTIR-ATR spectral fingerprints. The model highlights clear class discrimination, with each group forming well-defined clusters along the predictive (PLS1) and orthogonal (PLS2-PLS3) components

validating the model's robustness. The plant standards also remain isolated from all animal-based clusters, further demonstrating OPLS-DA's ability to differentiate plant-derived inputs from animal fats.

Meanwhile, the OPLS-DA model in Fig. 8 illustrates well-defined separation among all fermented food samples of *budu* (BD1, BD2, BD3, and BD4), *belacan* (BL1, BL2, BL3, and BL4), *pekasam* (P1), and *cincalok* (C1 and C2) with reference to the animal standard of lard, demonstrating strong discriminatory power of FTIR-ATR spectral power on the fingerprint region. Moreover, each fermented food product formed a coherent cluster, reflecting consistent intra-product lipid profiles characteristic of its respective raw materials and fermentation processes. In contrast, the lard standard was positioned distinctly away from all fermented food matrices along the predictive PLS1 axis. This marked separation is highly relevant to the scope of halal authentication, as it confirms that the fermented products do not exhibit spectral behavior resembling non-halal adulterants, such as lard in this case. Furthermore, the orthogonal components effectively captured the subtle variations among the different fermented products themselves, demonstrating the model's sensitivity in resolving biochemical differences arising from diverse substrates and microbial activity. From a halal perspective, these findings reinforce analytical confidence by providing evidence of product purity and supporting the assurance that no cross-contamination with non-halal fats occurs in raw materials, during processing, or in the end-products of fermented foods. The tight clustering patterns also highlight strong product consistency, aligning with quality and integrity.

### TPC, *S. aureus*, and *E. coli* Assessment

The microbiological quality of the food samples was assessed using three essential parameters: TPC, detection of *E. coli*, and identification of *S. aureus*. These results are presented in Table 3 within the context of comprehensive safety and quality. TPC serves as an indicator of the total number of viable microorganisms in a sample and is widely recognized for its role in assessing general hygiene

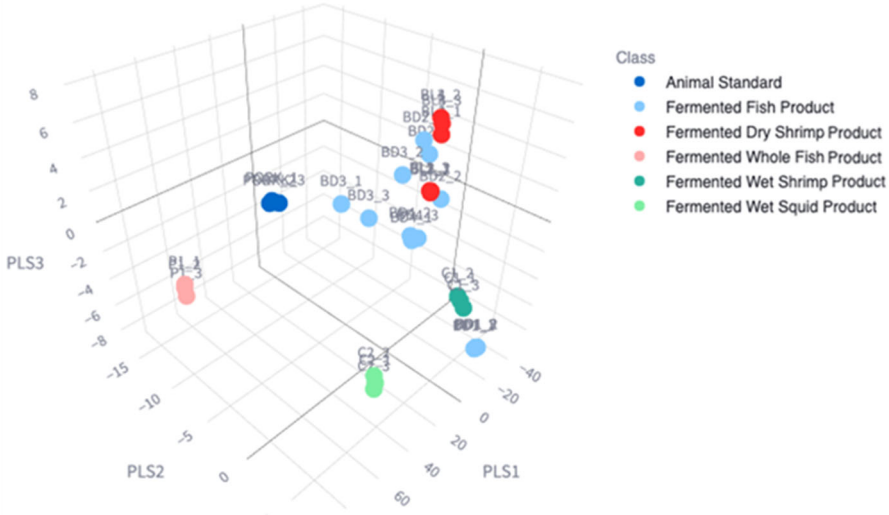


**Fig 8.** Three-dimensional OPLS-DA created with the Streamlit software framework score plot showing definite clustering of fermented food products of *budu* (BD1, BD2, BD3, and BD4), *belacan* (BL1, BL2, BL3, and BL4), *pekasam* (P1), and *cincalok* (C1 and C2) in clear separation from lard standard based on FTIR-ATR fingerprint region spectral data. The model demonstrates strong discriminatory power, distinguishing unique chemical profiles across sample groups for halal authentication

**Table 3.** The histamine concentration in animal adipose tissue and animal-based Malaysian fermented food products

| Sample | Total plate count (CFU/mL) | *E. coli* (CFU/mL/g) | *S. aureus* (CFU/mL/g) |
|---|---|---|---|
| BD1 | $2.34 \times 10^3$ | n.d | n.d |
| BD2 | $3.92 \times 10^2$ | n.d | $1.72 \times 10^2$ |
| BD3 | $1.24 \times 10^2$ | n.d | n.d |
| BD4 | $2.76 \times 10^2$ | n.d | $3.59 \times 10^3$ |
| BL1 | $5.35 \times 10^4$ | n.d | $3.23 \times 10^2$ |
| BL2 | $9.99 \times 10^3$ | n.d | $1.16 \times 10^3$ |
| BL3 | $7.62 \times 10^3$ | n.d | $1.16 \times 10^3$ |
| BL4 | $8.65 \times 10^3$ | n.d | $7.23 \times 10^3$ |
| P1 | $4.99 \times 10^3$ | n.d | n.d |
| C1 | $1.19 \times 10^3$ | n.d | $4.45 \times 10^2$ |
| C2 | $2.18 \times 10^3$ | n.d | $4.32 \times 10^2$ |

TPC quality control was using *E. coli* of value $6.59 \times 10^4$; n.d = not detected

and storage quality [36]. The TPC results from this study range from 1.24 × 102 (BD3) to 5.35 ($10^4$ CFU/mL (BL1), with variable levels across the eleven samples tested. According to the Malaysian Food Act 1983 and the Food Regulations 1985, particularly in the Fifteenth Schedule (Regulation 39), microbiological standards, including TPC limits, are specified for various food categories. Fermented meat products fall under RTE categories, where the TPC threshold permits up to $10^5$–$10^6$ CFU/g, reflecting the inherent microbial activity during controlled fermentation [37]. This study's TPC range remains below these limits, confirming compliance and indicating effective process controls under Section 13 of the Act, which prohibits the adulteration of unsafe food. Eventually, this range of microbial load also implies that differences in handling and processing practices substantially influence the results, respectively [37].

A particularly noteworthy finding is the complete absence of *E. coli* across all eleven samples tested. This result is highly significant for food safety assessment. *E.*

*coli* serves as an indicator organism for fecal contamination and poor sanitation practices in food processing. Moreover, the absence of *E. coli* across all samples demonstrates effective sanitation, hygiene controls, and appropriate processing conditions during the fermented food processing [38]. Moreover, the samples comply with the Malaysia Food Act 1985 on stringent pathogen controls in the Fifteenth Schedule, where *E. coli* must be absent or below detectable limits (<10 CFU/g) in most RTE food products. Section 18 of the Food Act empowers authorities to seize non-compliant products, but the complete absence here demonstrates adherence to the hygiene protocols outlined in Regulations 4 and 5 for microbiological sampling, thereby preventing the risk of fecal contamination.

*S. aureus* was detected in 7 of the 11 samples tested, with concentrations ranging from 1.72 ($10^2$ CFU/mL/g (BD2)) to 7.23 ($10^3$ CFU/mL/g (BL4). Notably, 4 samples of fermented food products showed no detectability of *S. aureus* (BD1, BD3, and P1). This variable presence pattern is important to understand in the context of the safety of fermented food products. Worth noting, *S. aureus* naturally occurs in meat environments and can be introduced during animal processing [39]. However, detection of *S. aureus* does not automatically indicate a safety risk in fermented food products. The organism can produce heat-stable enterotoxins, which are the primary concern for food safety. However, several factors in the production of fermented food products may inhibit toxin production. Most importantly, the result aligns with permissible levels in fermented food products, as the act focuses on toxin risks rather than total counts during processing, which might be influenced by factors such as low pH during fermentation or the addition of preservatives for storage [40]. Regulation 39 does not mandate zero tolerance for *S. aureus* in fermented food categories if toxin production is controlled, supporting the study's safety profile under section 10, which requires sanitary premises to mitigate staphylococcal enterotoxins.

## ■ CONCLUSION

This study demonstrates that a multi-dimensional analytical approach is valuable for evaluating the safety and halal permissibility of animal-based fermented food products. The combined application of histamine quantification, FTIR-ATR spectroscopy, microbiological assessment, and chemometric analysis successfully addressed both chemical safety and halal authentication objectives. Elevated histamine levels observed in a substantial proportion of samples highlight a critical vulnerability in traditionally Malaysian fermented food products, emphasizing the need for improved fermentation control and monitoring, particularly for regulatory authorities. In parallel, FTIR-ATR spectroscopy coupled with PCA and OPLS-DA demonstrated strong discriminatory capability, enabling clear differentiation between halal fermented food products and a non-halal reference (lard), thereby validating its potential as a rapid authentication tool. Microbiological findings further supported product acceptability by confirming compliance with hygienic quality requirements, thereby establishing a foundational framework integrating chemical, spectroscopic, and microbiological indicators for halal assessment of fermented food products. Moreover, this integrated approach provides a scientifically defensible methodology for quality control, regulatory enforcement, and halal assurance, offering a practical platform for routine screening and early detection of safety or authenticity risks. However, further validation across diverse fermented product types, larger sample cohorts, and multiple geographic regions would be necessary to develop standardized, industry-wide halal food parameters. Finally, this work contributes to the broader development of robust halal authentication methodologies and serves as a foundation for future comparative studies in halal food assessment.

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## ■ CONFLICT OF INTEREST

All authors declare that they have no known conflict of interest that could have influenced the work reported in this paper.

## ■ AUTHOR CONTRIBUTIONS

Muhammad Zulhelmi Nazri: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Visualization, Funding acquisition, Writing – Original draft. Siti Nor Azlina Abd Rashid, Nur Fashya Musa, Abd Rahman Jabir Mohd Din, Rozaliana Ab Karim, Hajar Aminah A. Karim, Salimah Ab Malik: Methodology, Formal analysis, Investigation, Data curation, Visualization, Writing – Original draft. Muhamad Shirwan Abdullah Sani, Dayang Norulfairuz Abang Zaidel, Hesham Ali El-Enshasy: Supervision, Validation, Writing – Review & Editing.

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