OPEN ACCESS

Al-Qālam القلم

P-ISSN: 2071-8683; E-ISSN: 2707-0077
Volume 30, Issue, 1, 2025
http://alqalamjournalpu.com/

Chemical Methods for Halal Food Authentication: From Theory to Practice

Faiq Haseeb
M.Phil scholar, Department of Chemistry,
University of Engineering & Technolgy,
Lahore

Abstract

Islamic dietary law requires the consumption of food that is both halal (permissible) and tayyib (pure and wholesome). Due to rising concerns over food adulteration and mislabeling, especially the accidental presence of pork or other haram substances, analytical methods have been developed to verify halal status. This review highlights key chemical and biochemical techniques, including PCR, GC-MS, FTIR, and ELISA, outlining their principles, instrumentation, applications, benefits, limitations, and case studies. A comparative table evaluates each method by specificity, sensitivity, cost, and sample suitability. The review also connects these scientific tools with Islamic teachings and discusses their implications for consumers, manufacturers, and certification bodies, offering a practical guide to halal food authentication.

KEYWORDS:
halal authentication; analytical chemistry; PCR; GC-MS; FTIR; ELISA; food adulteration; halal certification; Muslim consumer trust

Date of Publication:
30-06-2025

Introduction

Islamic dietary law requires that edible products be *halal* permitted and free from *haram* forbidden substances. The Quran explicitly enjoins believers to consume that which is lawful and good: *"O you who believe! Eat of the good things which We have provided for you"* Qur'an 2:172, Sahih International. By contrast, certain items pork and its by-products, blood, carrion, and intoxicants are categorically prohibited. For example, the Quran states that *"He has only forbidden you dead animals, blood, the flesh of swine..."* Al-Mā'idah 5:3 (Fathima et al., 2024). Thus, ensuring that food products are genuinely halal is not merely a cultural preference but a religious imperative for Muslims worldwide. In practice, the concept of halal often is paired with *tayyib*, meaning wholesome or pure, underscoring that halal foods must also meet standards of cleanliness and safety.

The global halal food market has grown substantially in recent years due to population growth and increasing demand in Muslim-majority and international markets. Halal products, especially meat and poultry, now represent a significant segment of the food industry together accounting for roughly 30% of global meat demand. Market analysts project continued growth e.g. a compound annual growth rate of ~6% into 2027. However, with growth comes risk: high demand and supply-chain complexity have led to incidents of fraud and contamination. Well-publicized scandals, such as the 2013 "beef" scandal in Europe, where beef burgers were found adulterated with pork and horse meat and numerous cases of mislabeling or improper handling have shaken consumer confidence and highlighted the need for rigorous authentication. In a recent review, Fathima *et al.* note that improper slaughter, mislabeling, adulteration, and contamination constitute the main non-compliance issues threatening halal integrity (Fathima et al., 2024).

With the growing availability of halal food, concerns regarding the authenticity of these products have increased among both Muslim and non-Muslim consumers (Kurniadi & Frediansyah, 2017). Halal certification has become essential, particularly for meat products, as they are a crucial component of a balanced human diet. Like food, medicines are also fundamental to human health, and with advancements in globalization, biochemistry, and industrialization, the demand for reliable and ethically produced products has surged.

Technological progress has significantly enhanced the field of food science, yet some manufacturers misuse these innovations by incorporating non-halal ingredients, misleading consumers. Mislabeling or incomplete information about ingredient origins is common in certain food products (Montowska & Pospiech, 2011). Some packaged items even lack halal certification altogether, raising questions about their permissibility and necessitating further investigation (Table 1).

Globally, meat and meat-based products are widely consumed due to their rich nutritional value, offering high-quality proteins, essential amino acids, vitamins, and minerals (Hossain et al., 2021). Gelatin, frequently found in food, medicine, and cosmetic items, is favored for its gelling, stabilizing, and healing properties, making it a popular component in commercial formulations (Mohd Zin et al., 2021).

Alcohol also plays a notable role in the pharmaceutical, food, and cosmetics industries. However, the inclusion of ethanol in products presents a challenge regarding its halal status, as many Islamic scholars consider ethanol to be haram (Khattak et al., 2011). Some beverages may contain trace amounts of alcohol, which must be properly identified to determine their compliance with halal standards (Alzeer & Abou Hadeed, 2016).

Traditionally, halal certification relies on regulatory oversight, documentation, and oversight by religious authorities. Yet these measures alone may not detect hidden adulterants or verify processing history. Analytical testing has therefore become an essential complement. Modern analytical chemistry and biotechnology offer tools to *"safeguard consumer trust and ensure adherence to religious dietary guidelines"*. This paper reviews several key chemical methods that have been applied for halal food authentication, discussing how each works and how it is used in practice (Fathima et al., 2024).

1.1. Islamic Dietary Context

In Islam, the lawful status of food is grounded in scripture and tradition. As one review explains, *"The term ḥalal implies an object that is not prohibited by religion"*, and the Quran and Hadith enumerate forbidden foods e.g. blood, pork, carrion that are *"not good for humans"*. Conversely, *tayyib* often translated as "pure" or "good" refers to food that is wholesome, balanced, and safe. The Quran highlights the unity of halal and tayyib: believers are encouraged to choose foods that are both lawful and nutritious. For example, the Prophet Muhammad is reported to have said that "innocuous" tayyib food promotes health, and scriptural verses e.g. Qur'an 7:31 admonish moderation and purity in eating. Thus, halal authentication not only concerns religious compliance but also broader standards of food quality and safety.

In Islam, foods that are not allowed, such as pork and alcoholic beverages, are classified as *haram*. In contrast, *halal* foods are those deemed permissible under Islamic law and are often labeled or certified by authorized halal certification agencies. To obtain such certification, food producers must meet specific requirements throughout all phases of the production process, including slaughtering, storage, processing, marketing, and hygiene practices. With Muslims making up roughly 23% of the global population, or around 1.6 billion people adherences to halal dietary standards holds significant importance for many. Practicing Muslims exclusively consume halal foods and studies show they generally have more favorable attitudes and intentions toward halal products compared to non-halal options. The global halal food market is valued at approximately USD 632 billion, representing around 16% of total global food consumption. While some major food companies such as Nestlé and Unilever have long offered halal-certified products, the restaurant and fast-food sectors have been relatively slow to cater to the halal market. As consumers become more informed and financially capable, they are increasingly focused on the integrity and traceability of the foods they consume. Religious beliefs continue to play a key role in shaping individual dietary habits and ethical responsibilities (Usman et al., 2024).

Halal certification bodies such as JAKIM in Malaysia, MUI in Indonesia, etc. apply these principles through regulations governing slaughter, processing, and ingredient sourcing. They often require traceability documentation and, increasingly, laboratory testing. As Spiegel *et al.* note, halal assurance in supply chains is achieved via audits and lab analysis to verify compliance (Van der Spiegel et al., 2012). In fact, consumers demand transparency: surveys indicate that Muslims are willing to pay a premium for halal certification, and any doubt about authenticity can damage brand trust. For example, Fathima *et al.* observe that incidents of fraud have *"ignited a heightened awareness among Muslim consumers ... regarding the significance of halal authenticity"(Fathima et al., 2024)*. Accordingly, reliable analytical methods are critical not only for producers and regulators but also for upholding the spiritual and ethical values that underpin halal food consumption.

In Islam, dietary laws emphasize the importance of consuming halal permissible food, which includes specific guidelines for animal slaughter. A key requirement is that the animal must be bled thoroughly, as the removal of blood is considered essential for purification. This method is not only a religious obligation but also ensures that the meat is fit for consumption according to Islamic principles. The slaughter involves cutting the throat, windpipe, and blood vessels in the neck with a sharp, clean knife while invoking the name of Allah. This practice is widely followed in Muslim-majority countries and is consistent with halal regulations that prioritize both hygiene and compassion during the process (Mortas et al. 2022).

1.2. Challenges and Objectives of Authentication

The wide variety of food products complicates halal testing. Adulterants may be introduced in raw materials e.g. inclusion of pork fat in beef products, during processing cross-contamination, or in labeling false claims of halal. Because pork and its derivatives gelatin, lard, collagen are among the most common adulterants in non-halal foods, many studies focus on detecting swine content. Likewise, the presence of undeclared alcohol or blood products can violate halal standards. Analytical challenges include: detecting very low levels of impurities in complex matrices; distinguishing between species in processed often heated foods; and handling products like gels or oils where the adulterant is chemically similar to permitted ingredients.

Various analytical strategies have been pursued. Conventional approaches include gross inspection color, texture, refractometry for alcohol, or dielectric property measurements (Usman et al., 2024). Modern analytical chemistry techniques are much more powerful. For example, DNA-based methods PCR and quantitative PCR can amplify minute amounts of species-specific DNA to reveal even trace pork contamination. Protein-based assays such as ELISA use antibodies to detect porcine proteins. Chromatographic and spectroscopic methods like GC-MS, HPLC, FTIR, NMR analyze chemical components, for instance, fatty acid profiles or volatile signatures, that differ between species. Advanced approaches even include metabolomics and biosensors (Usman et al., 2024), as well as CRISPR-based detection systems (Muflihah et al., 2023).

This review focuses on four widely used analytical methods: PCR, GC-MS, FTIR, and ELISA. For each we discuss the underlying principle, describe typical instrumentation, give examples of how it is applied to halal food testing often citing peer-reviewed case studies, and assess the method's strengths and weaknesses e.g. specificity, sensitivity, cost, sample types. A comparative summary table (Section 2.5) contrasts these techniques on key parameters. We also comment on real-world implementation: how these methods fit into the workflows of food manufacturers, testing laboratories, and halal certifiers. By linking theory with practice, we aim to provide a comprehensive resource for scientists, regulators, and industry stakeholders involved in halal food authentication.

2. Analytical Chemistry Methods for Halal Authentication

Analytical methods for detecting non-halal adulterants can be broadly classified by the type of analyte they target: DNA-based, protein-based, or chemical/physical. In halal contexts, DNA methods PCR are often used to detect species origin, especially pig DNA. Protein-based immunoassays ELISA detect animal proteins or epitopes e.g. porcine-specific collagen peptides. Chemical techniques chromatography, spectroscopy can analyze fats, oils, volatiles, or small molecules characteristic of certain species or processing methods **Error! Reference s ource not found.** compares key techniques. Below we review four principal methods in detail.

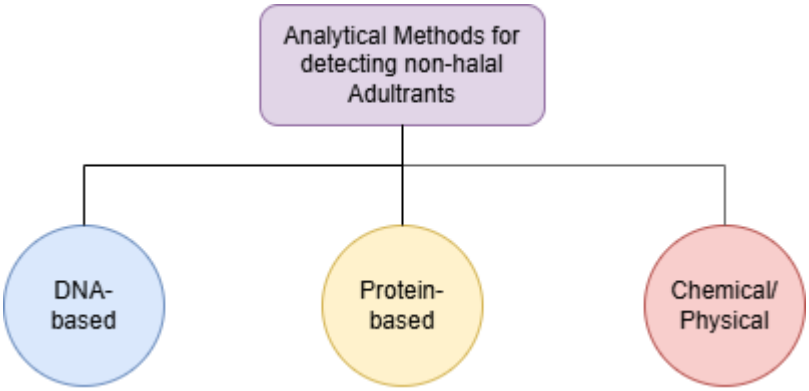

Figure 1 Analytical Methods for detection of non-halal adulterants

2.1. Polymerase Chain Reaction PCR

2.1.1. Principle and Instrumentation

Polymerase chain reaction PCR is a molecular biology technique for amplifying specific DNA sequences (Aprilia et al., 2022). In standard PCR, DNA is first extracted from a food sample. Species-specific primers short DNA sequences matching a target gene region are added along with DNA polymerase, nucleotides, and buffers. Thermal cycling repeated heating and cooling drives cycles of denaturation, annealing, and extension, doubling the amount of target DNA each cycle. After 30–40 cycles, even a few copies of a gene can be amplified to detectable levels. Real-time quantitative PCR qPCR adds fluorescent probes or intercalating

dyes to monitor amplification in real time, improving sensitivity and allowing quantification.

Instrumentation for PCR includes: a) a DNA extraction system chemical or kit-based to isolate pure DNA; b) a thermal cycler PCR machine to program precise temperature cycles; c) electrophoresis or real-time PCR instrumentation to detect amplicons. A typical laboratory setup might cost on the order of $10–50k PCR machine ~$10k, real-time PCR ~$20–50k. Some portable field PCR devices exist, but in halal testing use is mostly in well-equipped labs.

2.1.2 Application in Halal Authentication

PCR is widely used to detect porcine DNA in foods. Small tissue remnants muscle, skin, bone contain mitochondrial or nuclear DNA with sequences unique to pig. Primers targeting, for example, the porcine *cytochrome b* gene or mitochondrial control region have been validated. After DNA extraction, PCR amplifies even tiny amounts of pork DNA down to picogram levels in mixed samples (Aprilia et al., 2022). Agarose gel electrophoresis or melting curve analysis of qPCR confirms the presence of the pork-specific amplicon.

Case studies abound. For instance, Ali *et al.* 2012 developed a real-time PCR assay with a detection limit of ~0.001 ng pork DNA Muflihah et al., 2023 about 0.01% adulteration. Mandli *et al.* and others have similarly reported qPCR detection thresholds on the order of 0.1–1 pg DNA. In one study on Indonesian beef floss products dried shredded beef, a sandwich ELISA detected pork in 2 of 6 “halal” samples, whereas conventional PCR failed to amplify pork DNA in those samples. The authors suggested that heavy processing or heat might degrade DNA, making PCR less reliable than ELISA in that context. Conversely, if DNA is intact, PCR can be extremely sensitive. Lee *et al.* demonstrated detection of 0.001% pork in a raw meat mixture with qPCR targeting the porcine *beta-actin* gene Aprilia et al., 2022.

Multiplex PCR using several primer pairs simultaneously can test for multiple species at once e.g. pork, chicken, beef. High-Throughput variations e.g. TaqMan qPCR, digital PCR further improve accuracy and quantitation. Real-time PCR assays can be completed in ~1–2 hours once DNA is extracted. Data are quantitative, giving both presence and rough amount of contaminant. For example, Cheubong *et al.* (2014) detected pork adulteration at levels as low as 0.01% within 30 minutes using an optimized real-time PCR (Fathima et al., 2024).

2.1.3 Advantages and Limitations

Advantages: PCR offers extremely high specificity and sensitivity. Primers can discriminate single-nucleotide differences, virtually ensuring that a positive signal is from the target species. DNA persists in tissues even after extensive cooking or processing up to a point, so PCR can detect adulterants in cooked foods where organoleptic cues are lost. Once set up, many samples can be run in parallel in a well qPCR plates. Detection limits can reach low parts-per-million. PCR methods are generally regarded as gold-standard in species identification similar to forensic applications.

Limitations: PCR requires clean DNA, so samples with inhibitors spices, fats, heavy preservatives can yield false negatives. Intense heat or chemical processing can fragment DNA; very highly processed products e.g. gelatin may have insufficient intact DNA for amplification. PCR equipment and reagents add cost though not as high as MS instruments. There is risk of contamination in the lab careful controls are needed. Standard PCR detects presence/absence, and distinguishing trace levels from background requires careful calibration. Also, PCR targets specific known species adulteration by an unexpected species or one without specific primers would be missed unless a broad-spectrum analysis is done.

Real-world deployment: Many halal certification bodies and quality labs incorporate PCR. For example, the Malaysian Halal Development Corporation HDC recommends PCR testing for porcine DNA in meat products. Accredited test kits are available SYBR Green or TaqMan for rapid screening. However, PCR testing is typically done on suspicious or random samples due to cost and throughput constraints. For on-line rapid checks, portable PCR or isothermal amplification devices are emerging but not yet widespread.

2.2. Gas Chromatography–Mass Spectrometry GC-MS

2.2.1. Principle and Instrumentation

Gas chromatography–mass spectrometry combines separation and identification of volatile or semi-volatile compounds. In GC-MS, a food sample often after extraction or derivatization is vaporized in an inert gas stream like helium. The gas chromatograph separates compounds by volatility on a long capillary column, yielding different retention times. Eluted compounds then enter the mass spectrometer, where molecules are ionized often by electron impact and fragmented. The mass spectrometer detects the mass-to-charge ratios of fragments, producing a mass spectrum, a fingerprint that can identify a compound.

Typical GC-MS instrumentation is costly ~$50k–200k and involves a GC oven, columns, injector, and a mass analyzer quadrupole or ion trap. Sample preparation may include solvent extraction e.g. extracting fats into hexane or headspace analysis collecting volatiles above a sample. Derivatization e.g. methylation of fatty acids to FAMEs is often used to analyze oils and lipids.

2.2.2. Application in Halal Authentication

GC-MS is useful for analyzing chemical signatures that differ between animal species. For example, the fatty acid profile of lard pork fat differs from that of chicken or beef. Specific fatty acid markers have been identified: Sawaya *et al.* (1990) noted that the C20:2 polyunsaturated fatty acid is characteristic of pig fat Mortas et al., (2022), allowing GC profiling to detect as low as ~2% pork in mixed meat products. Similarly, gas chromatography can quantify steroid or lipid markers.

A practical application is testing oils and fats. Lestari *et al.* (2025) demonstrated the combined use of FTIR-ATR and GC-MS for detecting pork oil adulteration in halal fish oil (Lestari et al., 2025). In their study, fish oil halal was intentionally mixed with varying amounts of pork oil, and partial least squares PLS chemometrics on GC-MS data successfully quantified pork levels. They reported

100% correct classification of pure vs adulterated samples, identifying fatty acids like caprylic and pentadecanoic acid as markers. The conclusion was that **"**FTIR-ATR spectroscopy and GC-MS coupled with chemometrics is a rapid and accurate method for detecting and quantifying pork oil" (Lestari et al., 2025). In other words, a GC-MS analysis of fatty acid methyl esters FAMEs in the oil reliably flagged non-halal adulteration.

GC-MS is also used for meat and processed foods by headspace analysis of volatiles. Nurjuliana *et al.* (2011) applied headspace GC-MS to raw and smoked meats. They collected volatile organics from ground pork, beef, chicken, and their sausages, then applied principal component analysis to the spectral data. The GC-MS with headspace sampling achieved distinct clustering: pork samples formed a separate group from beef, mutton, and chicken (Nurjuliana et al., 2011). This shows that complex odor profiles can fingerprint species: even without DNA or proteins, GC-MS detected specific volatiles e.g. 2-pentyl furan, aldehydes that were abundant in pork. Thus, GC-MS *could* be used to identify even cooked or processed meats based on aroma compounds, supplementing other tests.

2.2.3. Advantages and Limitations

Advantages: GC-MS is highly specific in chemical identification. A mass spectrum can unambiguously indicate a compound given spectral library. It can detect multiple compounds simultaneously, providing a chemical "fingerprint." Quantitative results are possible with standards, and even trace levels ppm can often be measured. GC-MS can analyze non-biological markers e.g. lipid profiles, contaminants that PCR or ELISA cannot. It is versatile: by changing sample prep derivatization, headspace, one can target fatty acids, steroids, alcohols, or other volatiles.

Limitations: The equipment is expensive and requires skilled operators. Sample preparation can be laborious e.g. lipid extraction, methylation. Non-volatile or thermally labile compounds require derivatization adding cost/time. Not all targets are easily volatile e.g. large proteins. GC-MS sensitivity can be high, but if an adulterant is chemically similar e.g. pork fat vs beef fat, distinguishing them may require subtle analysis and chemometrics. For example, if small amounts of lard are mixed into other fat, the C20:2 marker may be diluted below the 2% detection threshold (Mortas et al., 2022). Also, interpreting complex GC-MS data demands computational analysis PCA, PLS, and there is potential for overlapping peaks. Real-case use: GC-MS is a standard in many food testing labs for contaminant analysis, residue monitoring, etc. For halal purposes, some labs use GC-MS to test for prohibited alcohols e.g. ethyl alcohol levels. GC-MS is also cited by certification guidelines for complex adulterants. However, routine use is often limited by cost and turnaround time; it is more common to use GC-MS in confirmatory or research settings rather than every batch of product

2.3. Fourier-Transform Infrared Spectroscopy FTIR

2.3.1. Principle and Instrumentation

FTIR spectroscopy measures how a sample absorbs infrared light at different wavelengths wavenumbers, generating a spectrum that reflects molecular vibrations

bonds in the material. Each chemical bond C–H, C=O, N–H, etc. has characteristic IR absorbance. Thus, complex materials produce a "fingerprint" spectrum. The FTIR instrument uses an interferometer and Fourier transform to convert raw signal to spectrum quickly.

In practice, an attenuated total reflectance ATR accessory is often used for foods. ATR-FTIR involves placing a small sample solid, liquid, or paste directly onto a crystal; IR light internally reflects through the crystal and penetrates the sample by a few microns. The advantages are minimal preparation no KBr pellet or thin film needed and rapid data collection seconds to minutes. FTIR spectrometers are moderately priced ~$15k–30k for an ATR system and easy to operate. Coupling FTIR with multivariate chemometric analysis PCA, PLS often enhances discrimination and quantitation.

2.3.2. Application in Halal Authentication

FTIR is used to differentiate fats, oils, proteins, and other food components based on spectral differences. For example, the IR spectrum of pork fat shows subtle differences in CH stretching or carbonyl peaks compared to chicken fat. Likewise, gelatin denatured collagen from different species has distinct amide band signatures. By analyzing the spectrum, one can detect adulteration or mixing.

One application is gelatin authentication. Hassan *et al.* (2025) developed an ATR-FTIR method to quantify pork gelatin contamination in bovine gelatin. They took advantage of how ethanol affects the IR absorbance of gelatin amide bands differently for pork vs beef. With a calibration curve, the method achieved a strong linear correlation $R^2 = 0.9994$ between pork content and amide band intensity. The detection limit was 0.85 mg pork per 100 mg gelatin 0.85% with quantification at 2.85 mg/100 mg. This sensitivity allowed them to identify pork gelatin even in halal gummy candy, with recoveries of 50–104%. Thus, ATR-FTIR proved a *rapid, accurate approach* for gelatin source identification (Hassan et al., 2025).

FTIR has also been applied to fats and oils as in Lestari's study mentioned above. In that work, FTIR-ATR combined with chemometric LDA/PLS achieved 100% classification of fish oil vs pork oil vs mixtures Lestari et al., 2025. In practice, one would measure the IR spectrum of a lipid sample typically 4000–800 cm^{-1} range and use a trained model to predict adulteration. Advantages include no need for solvents or extensive prep, and analysis time of a few minutes. FTIR is also non-destructive, so samples remain available for further testing if needed.

In a broader halal context, FTIR has been used to classify meat types and detect adulteration. Cebi *et al.* (2016, 2019) and others have shown that ATR-FTIR spectra of gelatin or emulsified meats can be differentiated by species. For example, spectra of porcine, bovine, and fish gelatins form separable clusters under PCA (Hassan et al., 2025). Such approaches are promising for on-site or quick checks, especially when coupled with portable IR spectrometers.

2.3.3. Advantages and Limitations

Advantages: FTIR is rapid seconds–minutes per sample and requires little sample preparation. It is relatively inexpensive per sample no consumables, no solvents, and

the equipment costs are moderate. ATR-FTIR is nondestructive and can analyze solids, liquids, and semi-solids. The technique is "universal" in the sense that most organic substances absorb IR; one spectrum can reveal multiple components at once. With chemometric tools, FTIR can often achieve high classification accuracy as noted, 100% in the cited studies (Lestari et al., 2025). It is suitable for routine screening in quality control settings.

Limitations: FTIR's specificity is lower than molecular methods. Similar compounds can give overlapping spectral features, making it sometimes hard to pinpoint a unique marker without multivariate analysis. Interpretation usually requires comparison with reference spectra or statistical models built from known samples. Sensitivity is moderate: in Hassan's gelatin study, LOD was ~0.85% pork (Hassan et al., 2025). Trace adulterations below ~1% may escape detection. Also, water strongly absorbs IR in the 3200–3600 cm^{-1} range, so highly hydrated samples can complicate analysis. Finally, building a robust FTIR model requires a good training set of authentic and adulterated samples; without that, predictions may be unreliable. In practice, FTIR is often used in conjunction with other methods. For example, a positive FTIR screen for pork oil might be followed by confirmatory PCR or GC-MS. Nonetheless, its speed and low cost make FTIR attractive for preliminary checks, especially for fat/oil and gelatin products where quick turnaround is needed.

2.4. Enzyme-Linked Immunosorbent Assay ELISA

2.4.1. Principle and Instrumentation

ELISA is an antibody-based assay for detecting specific proteins or peptides. In a typical sandwich ELISA for meat species detection, one uses antibodies that bind to an animal-specific protein e.g. porcine serum albumin or myosin. A food sample is first homogenized/extracted in a buffer; the extract is applied to a microplate well coated with a capture antibody. Any target protein from pork binds to the antibody. After washing, a second enzyme-linked antibody specific to the target is added, forming a "sandwich." A substrate is then introduced that the enzyme converts to a colored or fluorescent product. The intensity of the signal measured on a plate reader correlates with the amount of target antigen present.

Commercial ELISA kits for pork detection are available from various suppliers. These kits use standardized reagents and protocols for sample preparation, incubation, and detection. Equipment needs are modest: a blender/homogenizer for sample prep, incubator, and an ELISA plate reader absorbance microplate reader, usually ~$5–10k. The per-test cost is relatively low a kit can test many samples, and turnaround is hours rather than days.

2.4.2. Application in Halal Authentication

ELISA is widely used to screen for porcine proteins in foods such as gelatin-containing products, sausages, and mixed-meat dishes. For example, an ELISA kit targeting porcine myoglobin or hemoglobin can confirm the presence of even denatured proteins from pork. The technique can work on cooked meats, since many protein epitopes survive heat unlike DNA, which can be degraded.

In one study on halal beef floss a dried meat product, researchers compared a porcine ELISA kit to PCR. The ELISA detected pork in 2 out of 6 samples OD values significantly above cutoff. The same samples gave no band by conventional PCR, suggesting the DNA was too degraded for that assay. The authors concluded that *"ELISA is better than conventional PCR method for product samples that have received an intensive heating process"*. In another independent study by Yörük et al. (2021), comparison of different commercial kits found ELISA had comparable sensitivity to real-time PCR for detecting pork in meat mixtures down to ~0.1% level (Aprilia et al., 2022).

ELISA has also been used to validate halal claims in processed foods globally. Kuswandi *et al.* (2017) used a porcine gelatin ELISA to detect pork-derived gelatin in Indonesian noodles. Taha *et al.* (2018) applied an ELISA to test candies for pork gelatin. In these cases, the ELISA could flag traces of pork gelatin that manufacturers had not labeled. Overall, ELISA is considered a practical method for routine screening: it can handle many samples in parallel 96-well format with results in a few hours.

2.4.3 Advantages and Limitations

Advantages: ELISA kits are user-friendly, relatively inexpensive, and quick. They do not require specialized training beyond basic lab skills. Because they target proteins, they can sometimes detect species markers even if DNA is heavily degraded as long as the epitope survives. ELISA results are quantitative via standard curves and can yield detection limits around parts per thousand or better. The method can also be very specific if the antibody is well-selected e.g. raised against a unique porcine peptide .

Limitations: Like any protein assay, ELISA depends on the availability and integrity of antigen. Highly processed foods e.g. those subjected to extreme heat, pH, or enzymatic treatment might denature or remove the target proteins, causing false negatives. Cross-reactivity is another concern: poorly characterized antibodies might react weakly with non-target species, though good kits minimize this. ELISA cannot identify the source of adulteration beyond what it is designed to detect e.g. a pork ELISA won't catch chicken adulterant. The test is destructive sample is consumed and semi-quantitative at best unless rigorously calibrated. Finally, ELISA requires an initial expense for a plate reader, but that is generally lower than for PCR or MS instruments. In practice, ELISA is often used for high-throughput monitoring. Halal certifiers may require ELISA testing especially for gelatin and hydrolyzed products. Some regulatory bodies include ELISA protocols in their standards. Overall, ELISA provides a balance of ease and performance: faster and simpler than DNA tests, and more direct than physical inspections.

2.5. Comparative Analysis of Methods

Error! Reference source not found. summarizes and compares PCR, GC-M S, FTIR, and ELISA for halal food authentication across several criteria: specificity, sensitivity detection limit, cost, and typical sample types. These categories illustrate the trade-offs each method entails.

Each method has niche strengths. PCR's unmatched specificity allows definitive identification of pig DNA e.g. cyt*b* or rRNA genes, making false positives misidentifying the wrong species extremely unlikely. Its sensitivity can reach nanogram or even picogram levels of DNA (Aprilia et al., 2022). However, PCR does require effective DNA extraction, which can be inhibited by complex food matrices. GC-MS excels at chemical profiling. For example, the C20:2 fatty acid marker enables pork fat detection down to a few percent (Mortas et al., 2022). Volatile GC-MS headspace can cluster pork vs other meats (Nurjuliana et al., 2011). The high cost of equipment and sample prep is balanced by rich data output. In halal labs, GC-MS is often used for specific applications e.g. confirming adulteration found by screening, or for oils and spices where species DNA is absent

FTIR is fast and cost-effective. As reported, ATR-FTIR spectrometry could quantify pork gelatin at ~0.85% levels Hassan et al., (2025) and classify pure vs adulterated fats with 100% accuracy when combined with chemometrics (Lestari et al., 2025). Its sensitivity is lower than PCR: trace contaminants below ~0.5% might go undetected. But for routine checks of raw or simple products, FTIR is attractive due to minimal consumables.

ELISA offers a balance: good specificity for a protein antigen, and reasonably high sensitivity. In practice, ELISA kits for pork can detect on the order of 1–5 ng protein per mL of extract roughly corresponding to ~0.1% in meat Aprilia et al., 2022. Its speed plate-based assays and ease make it suitable for batch testing. ELISA is especially common in halal testing for gelatin and cooked meats, where DNA might be compromised but protein markers persist.

In summary, no single method covers all situations. PCR is preferred for raw meats and when ultimate sensitivity is needed. GC-MS is chosen for chemical analysis lipids, volatiles and high-specificity confirmatory tests. FTIR is used for rapid screening, often in combination with chemometrics. ELISA is used for processed meats and gelatin screening. Table 1 provides an at-a-glance guide; laboratories often use a combination e.g. FTIR or ELISA for screening, with PCR or GC-MS for confirmation.

Method	PCR qPCR	FTIR-ATR	ELISA
Target	DNA sequences	Molecular functional groups	Proteins/peptides antigen
Specificity	Very high species-specific primers	Moderate global fingerprint + chemometrics	High antibody-specific
Sensitivity LOD	Very high down to ~0.01–0.1% contamination;	Moderate typically ~0.5–1% adulterant	High percent to sub-percent; e.g. ~0.1% possible
Cost	$$ thermal cycler	$–$$ instrument, chemometrics	$$ plate reader, kits
Sample Type	Meat raw or cooked, processed after DNA extraction	Oils/fats, proteins, gelatin, mixtures pressed liquid	Meat extracts, processed products, gelatin, sausages
	Aprilia et al., 2022	Hassan et al., 2025	Aprilia et al., 2022

Table 1 Comparison of analytical methods for halal authentication. Specificity and sensitivity are relative e.g. " very high" ~ species-specific, " high" ~ detection in low percentages. Cost ranges are approximate $=low, $$$=high

3. Implications for Stakeholders

The choice and deployment of authentication methods have important implications for various stakeholders in the halal food system.

3.1. Muslim Consumers

For Muslim consumers, the primary concern is assurance that products labeled "halal" truly meet religious standards. Analytical methods help guarantee this assurance. When independent testing or recall of misbranded products uses these techniques, consumer trust is reinforced. As seen from recent incidents, awareness has increased: consumers are vigilant and sometimes demand transparency regarding testing protocols. Ethical Muslims may also prefer brands that use scientific certification methods. However, it is also important to communicate that a certified halal mark backed by rigorous testing rather than mere trust is the highest assurance. Certification is often considered a "mark of quality" that affects purchase decisions, as studies on consumer behavior show e.g. higher trust and brand loyalty for certified products (Al Mustaqim et al., n.d.).

Conversely, use of these methods could raise consumer costs, as manufacturers may pass along testing expenses. But given the intangible value of trust in religious compliance, many consumers are willing to accept a modest price premium for certainty. In the long run, widespread adoption of robust testing protocols can elevate the overall reliability of halal products in the market, benefiting consumers.

3.2. Food Manufacturers and Importers

Manufacturers of meat, gelatin, sauces, and any processed food with halal claims must ensure supply-chain integrity. Analytical testing provides a way to audit suppliers and validate ingredients. Large companies often integrate such testing in their quality control QC labs or through third-party testing services. For example, a sausage manufacturer might PCR-test raw materials, GC-MS-test fat sources, or random-sample production runs with ELISA. These measures help avoid costly recalls and brand damage.

From a practical standpoint, implementing chemical authentication requires investment: training staff, validating methods, and possibly changing supplier specifications only using ingredients that test clean. This can be burdensome for small producers. Consequently, many smaller firms rely on certified suppliers or certifications of their own vendors. Even so, over the past decade there is a trend toward in-house testing in larger halal brands, particularly in markets like Malaysia, Indonesia, Middle East, and increasingly Western Muslim communities.

Regulators and certification bodies also encourage manufacturing oversight. For example, Indonesia's MUI and Malaysia's JAKIM have guidelines that recognize laboratory testing often in accredited labs as part of the halal audit. In practice, a manufacturer seeking certification may present lab test reports e.g. PCR results to demonstrate that no pork residues are present. Thus, chemical methods are becoming embedded in the halal compliance process.

3.3. Halal Certification Bodies and Regulators

Halal certification agencies are tasked with upholding religious standards. In recent years, many have emphasized formalizing the certification scheme to include scientific verification. As noted by Fathima et al. 2024, reliance "solely on physical examinations, documentation, and sharia expertise may not provide a comprehensive assessment" of compliance. Therefore, certifiers increasingly require that critical control points include laboratory checks. Some bodies even operate their own labs or designate approved external labs for testing. For instance, the Indonesian Food and Drug Monitoring Agency BPOM complements MUI by checking food safety and composition.

For certifiers, analytical methods enable objective decisions. Rather than a product being declared halal just by labeling or paperwork, test results can be cited. In disputes or consumer complaints, laboratory evidence is compelling. Certification bodies also participate in ring trials and method validation studies, to ensure testing consistency. Internationally, there are moves toward harmonizing halal testing standards analogous to ISO for food safety. However, challenges remain: different countries have different accepted lists of tests, and there is no single global protocol. In practice, many certifiers accept a suite of methods; some even allow non-analytical checks e.g. DNA barcoding vs. biochemical.

Overall, the implication is a shift toward evidence-based certification. This raises the bar for halal integrity but also for overhead: certifiers must oversee high-tech lab networks. On the positive side, robust authentication reinforces the credibility of halal certification as a whole.

4. Conclusion

Advances in analytical chemistry have transformed halal food authentication from an informal assurance into a precise science. Techniques such as PCR, GC-MS, FTIR, and ELISA enable detection of forbidden ingredients especially pork at trace levels, thus operationalizing the Islamic injunction to consume "good and pure" foods. Each method has unique strengths: PCR excels at sensitive DNA identification; GC-MS provides detailed chemical profiling; FTIR offers rapid fingerprinting; ELISA gives user-friendly protein detection. In practice, these methods are often used together to achieve both broad screening and confirmatory testing.

This paper has outlined the theory and practice behind these chemical approaches. We have drawn on recent peer-reviewed studies to illustrate how these methods are applied to real cases e.g. gelatin analysis, oil adulteration, meat mixtures. The comparative table highlights trade-offs between specificity, sensitivity, and cost. Importantly, we underscore that halal authenticity is not just a laboratory issue, but a matter of consumer trust, industry practices, and certification policy. As discussed, the Quranic commandments on halal and tayyib provide the underlying mandate, and modern science offers the tools to uphold it.

Future directions include development of faster, portable assays e.g. LAMP, CRISPR-based biosensors and integrated approaches food metabolomics.

Continuous collaboration between religious scholars, scientists, and regulators will be needed to adapt these technologies to evolving food systems. Ultimately, the goal is to ensure that every Muslim can consume halal food with confidence, a goal that merges spiritual principle with scientific innovation.

Bibliography

Al Mustaqim, D., Fazriani, D., Hukum, J., & Syariah, E. (n.d.). *ANALYSIS OF THE EFFECT OF HALAL CERTIFICATION ON CONSUMER TRUST AND BRAND LOYALTY*.

Alzeer, J., & Abou Hadeed, K. (2016). Ethanol and its Halal status in food industries. *Trends in Food Science & Technology*, *58*. https://doi.org/10.1016/j.tifs.2016.10.018

Aprilia, P., Ummami, R., Airin, M., Aziz, F., & Astuti, P. (2022). Comparison of ELISA and PCR Assays for Detection of Pork Adulteration in Halal-Labelled Beef Products. *Journal of Food Quality and Hazards Control*, *9*. https://doi.org/10.18502/jfqhc.9.2.10648

Fathima, A. M., Rahmawati, L., Windarsih, A., & Suratno. (2024). Advanced Halal Authentication Methods and Technology for Addressing Non-Compliance Concerns in Halal Meat and Meat Products Supply Chain: A Review. In *Food Science of Animal Resources* (Vol. 44, Issue 6, pp. 1195–1212). Korean Society for Food Science of Animal Resources. https://doi.org/10.5851/kosfa.2024.e75

Hassan, H. M., Souka, U. D., & Hassan, S. M. (2025). Differentiation and quantification of bovine and pork gelatin using UPLC-QTOF and ATR-FTIR spectroscopy: Addressing challenges in mixed gelatin analysis and detection. *Food Chemistry*, *464*, 141883. https://doi.org/https://doi.org/10.1016/j.foodchem.2024.141883

Hossain, M. A. M., Uddin, S. M. K., Sultana, S., Wahab, Y. A., Sagadevan, S., Johan, M. R., & Ali, Md. E. (2021). Authentication of Halal and Kosher meat and meat products: Analytical approaches, current progresses and future prospects. *Critical Reviews in Food Science and Nutrition*, *62*(2), 285–310. https://doi.org/10.1080/10408398.2020.1814691

Khattak, J., Mir, A., Anwar, Z., Wahedi, H., Abbas, G., Khattak, H., & Ismatullah, H. (2011). Concept of Halal Food and Biotechnology. *Advance Journal Of Food Science & Emerging Technologies*.

Kurniadi, M., & Frediansyah, A. (2017). Halal Perspective of Microbial Bioprocess Based-Food Products. *REAKTOR*, *16*(3), 147. https://doi.org/10.14710/reaktor.16.3.147-160

Lestari, D., Hamzah, H., Dewi Pramesthi, A. D. E., Syamsul, E. S., Safitri, P. Dela, Nurjunnah, R., & Rohman, A. (2025). The employment of FTIR-ATR spectroscopy and GC-MS combined with chemometrics for rapid detection of adulteration of pork oil in Gabus fish oil (Channa striata) for halal authentication. *Open Veterinary Journal*, *15*(2), 646–659. https://doi.org/10.5455/OVJ.2025.v15.i2.13

Mohd Zin, Z., Sarbon, N., Zainol, K., Jaafar, S., Shukri, M., & ab rahman, A. (2021). *Halal and Non-Halal Gelatine as a Potential Animal By-Products in Food Systems: Prospects and Challenges for Muslim Community*. https://doi.org/10.2991/assehr.k.210312.086

Montowska, M., & Pospiech, E. (2011). Authenticity Determination of Meat and Meat Products on the Protein and DNA Basis. *Food Reviews International*, *27*. https://doi.org/10.1080/87559129.2010.518297

Mortas, M., Awad, N., & Ayvaz, H. (2022). Adulteration detection technologies used for halal/kosher food products: an overview. *Discover Food*, *2*(1), 15. https://doi.org/10.1007/s44187-022-00015-7

Muflihah, Hardianto, A., Kusumaningtyas, P., Prabowo, S., & Hartati, Y. W. (2023). DNA-based detection of pork content in food. *Heliyon*, *9*(3), e14418. https://doi.org/https://doi.org/10.1016/j.heliyon.2023.e14418

Nurjuliana, M., Che Man, Y. B., Mat Hashim, D., & Mohamed, A. K. S. (2011). Rapid identification of pork for halal authentication using the electronic nose and gas chromatography mass spectrometer with headspace analyzer. *Meat Science*, *88*(4), 638–644. https://doi.org/https://doi.org/10.1016/j.meatsci.2011.02.022

Usman, I., Sana, S., Afzaal, M., Imran, A., Saeed, F., Ahmed, A., Shah, Y. A., Munir, M., Ateeq, H., Afzal, A., Azam, I., Ejaz, A., Nayik, G. A., & Khan, M. R. (2024). Advances and challenges in conventional and modern techniques for halal food authentication: A review. In *Food Science and Nutrition* (Vol. 12, Issue 3, pp. 1430–1443). John Wiley and Sons Inc. https://doi.org/10.1002/fsn3.3870

Van der Spiegel, M., Van der Fels-Klerx, H. J. (Ine), Sterrenburg, P., Ruth, S., Scholtens, I. M. J., & Kok, E. (2012). Halal assurance in food supply chains: Verification of halal certificates using audits and laboratory analysis. *Trends in Food Science & Technology*, *27*, 109–119. https://doi.org/10.1016/j.tifs.2012.04.005